การแยก ลักษณะสมบัติและการแสดงออกของยีน และโปรตีนในอัณฑะของกุ้งกุลาดำ *Penaeus monodon*

นางสาว ศศิธร เพชรก้อน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ISOLATION, CHARACTERIZATION AND EXPRESSION OF GENES AND PROTEINS IN TESTES OF THE GIANT TIGER SHRIMP *Penaeus monodon*

Miss Sasithon Petkon

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By	Miss Sasithon Petkon
Field of Study	Biotechnology
Thesis Advisor	Professor Piamsak Menasveta, Ph.D.
Thesis Co-Advisor	Sirawut Klinbunga, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

..... Chairman

(Associate Professor Sirirat Rengpipat, Ph.D.)

..... Thesis Advisor

(Professor Piamsak Menasveta, Ph.D.)

..... Thesis Co-Advisor

(Sirawut Klinbunga, Ph.D.)

..... Examiner

(Associate Professor Somkiat Piyatiratitivorakul, Ph.D.)

..... External Examiner

(Sittiruk Roytrakul, Ph.D.)

ศศิธร เพชรก้อน : การแยก ลักษณะสมบัติและการแสดงออกของยีน และโปรตีนในอัณฑะของกุ้งกุลาดำ *Penaeus monodon* (ISOLATION, CHARACTERIZATION AND EXPRESSION OF GENES AND PROTEINS IN TESTES OF THE GIANT TIGER SHRIMP *Penaeus monodon*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ศ.ดร. เปี่ยมศักดิ์ เมนะเศวต, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร.ศิราวุธ กลิ่นบุหงา, 190 หน้า.

วิเคราะห์โปรติโอมิกส์ของโปรตีนทั้งหมดในอัณฑะของพ่อพันธุ์กุ้งกุลาดำที่มาจากธรรมชาติ (กลุ่ม A) และจากบ่อเลี้ยงอายุ14 เดือน (กลุ่ม B และกลุ่ม C) ด้วยวิธี two-dimensional gel electrophoresis (2-DE) จากนั้นทำการแยกวิเคราะห์จุดโปรตีนในอัณฑะของกุ้งกุลาดำโดยใช้เทคนิค nanoLC-MS/MS โดยวิเคราะห์จุดโปรตีนในอัณฑะของกุ้งกุลาดำจำนวนทั้งสิ้น 640 จุดโปรตีน ประกอบด้วย 394, 120 และ 126 จุดโปรตีนในกุ้งพ่อพันธุ์ธรรมชาติกลุ่ม A กุ้งพ่อพันธุ์เลี้ยงอายุ14 เดือน กลุ่ม B และกลุ่ม C ตามลำดับ โดยพบโปรตีนที่น่าสนใจ เช่น farnesoic acid-O-methyltransferase (FAMeT), progesterone receptor-related protein p23, receptor activating protein kinase C (RACK), 14-3-3-like protein และ NADP-dependent leukotriene B4 12-hydroxydehydrogenase (LTB4DH) เป็นต้น

นอกจากนี้ได้ศึกษาโปรติโอมิกส์ของโปรตีนทั้งหมดในอัณฑะของพ่อพันธุ์กุ้งกุลาดำกลุ่มต่างๆ ประกอบด้วย พ่อพันธุ์กุ้งที่มาจากธรรมชาติ (กลุ่ม A และ กลุ่ม B) และพ่อพันธุ์กุ้งจากบ่อเลี้ยงอายุ14 เดือน (กลุ่ม C) และอายุ18 เดือน (กลุ่ม D) ด้วยวิธี one-dimensional del electrophoresis (1-DE) จากนั้นทำการแยกวิเคราะห์โปรตีนทั้งหมดตามช่วงขนาดโมเลกุล โดยใช้เทคนิค nanoLC-MS/MS พิจารณาโปรตีนประมาณ 50 ตัวแรกที่มีการแสดงออกที่มากขึ้นหรือน้อยลงอย่างชัดเจนในแต่ละช่วงขนาดโมเลกุล พบโปรตีนทั้งหมดจำนวน 345 โปรตีน โดยจัดเป็นโปรตีนที่พบเฉพาะในอัณฑะของกุ้งพ่อพันธุ์ธรรมชาติกลุ่ม A จำนวน 1 (0.29%) โปรตีน โปรตีนที่พบเฉพาะในอัณฑะของกุ้งพ่อพันธุ์ธรรมชาติทั้งกลุ่ม A และ B จำนวน 18 (5.22%) โปรตีน และโปรตีนที่พบในอัณฑะของกุ้งทุกกลุ่มจำนวน 231 (66.96%) โปรตีน โดยพบโปรตีนที่น่าสนใจ เช่น vasa-like protein, Ran GTPase activating protein 1 และ seven transmembrane helix receptor เป็นต้น

หาลำดับนิวคลีโอไทด์ที่สมบูรณ์ของยีนต่างๆโดยเทคนิค RACE-PCR พบว่า *ubiquitin specific peptidase 14, ubiquitin carboxyl-terminal hydrolase 5, Cdk17.* และ *proteasome alpha subunit, putative* มี open reading frame (ORF) ขนาด 1524, 2442, 1470 และ 765 คู่เบส แปลรหัสได้เป็นโปรตีนขนาด 507, 813, 489 และ254 กรดอะมิโน ตามลำดับ เมื่อตรวจสอบการแสดงออกของยีนเหล่านี้ในเนื้อเยื่อต่างๆของกุ้งพ่อพันธุ์เพศผู้และในรังไข่ของกุ้งแม่พันธุ์เพศเมีย พบว่ายีนต่างๆมีการแสดงออกในทุกเนื้อเยื่อที่ทำการศึกษา

เมื่อศึกษาการแสดงออกของยีนต่างๆในอัณฑะของกุ้งกุลาดำเพศผู้ด้วยวิธี quantitative real-time PCR พบว่า *serine/threonine protein kinase 23* และ *ubiquitin specific peptidase 14* มีการแสดงออกที่ไม่แตกต่างกันระหว่างกุ้งวัยรุ่นอายุ 6 เดือน และกุ้งพ่อพันธุ์อายุ 10 เดือน 14 เดือน และ 18 เดือน รวมทั้งกุ้งพ่อพันธุ์จากธรรมชาติ ($P > 0.05$) ส่วน *proteasome alpha* subunit และ *proteasome delta* ในกุ้งเลี้ยงอายุ 10 เดือน และ 14 เดือน มีการแสดงออกไม่แตกต่างจากกุ้งพ่อพันธุ์ธรรมชาติ ($P > 0.05$) แต่มีการแสดงออกที่สูงกว่ากุ้งวัยรุ่นอายุ 6 เดือน และกุ้งพ่อพันธุ์อายุ 18 เดือนอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) นอกจากนี้ *26S proteasome regulatory subunit S3* ยังมีการแสดงออกในอัณฑะของกุ้งพ่อพันธุ์อายุ 14 เดือนสูงกว่ากุ้งวัยรุ่นอายุ 6 เดือน กุ้งเต็มวัยอายุ 10 เดือน และ 18 เดือน อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) แต่ไม่แตกต่างจากกุ้งเต็มวัยจากธรรมชาติ ($P > 0.05$) ผลจากการทดลองบ่งชี้ว่า กุ้งตัดพันธุ์เพศผู้ที่ทำการเลี้ยงเริ่มสมบูรณ์พันธุ์เมื่ออายุ 10 เดือน โดยน่าจะมีความสมบูรณ์พันธุ์สูงสุดที่อายุ 14 เดือน และมีความความสมบูรณ์พันธุ์ลดลงที่อายุ 18 เดือน

สาขาวิชา.....เทคโนโลยีชีวภาพ..... ลายมือชื่อนิสิต.....ศศิธร เพชรก้อน.....

ปีการศึกษา.....2552..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5072480823 : MAJOR BIOTECHNOLOGY
KEYWORDS : *Penaeus monodon* / GIANT TIGER SHRIMP / TWO-DIMENSIONAL GEL ELECTROPHORESIS / MASS SPECTROMETRY

SASITHON PETKON: ISOLATION, CHARACTERIZATION AND EXPRESSION OF GENES AND PROTEINS IN TESTES OF THE GIANT TIGER SHRIMP *Penaeus monodon*. THESIS ADVISOR: PROF. PIAMSAK MENASVETA, Ph.D, THESIS CO-ADVISOR: SIRAWUT KLINBUNGA, Ph.D, 190 pp.

Proteomic analysis based on two-dimensional gel electrophoresis (2-DE) was carried out to identify reproduction-related proteins in testes of wild and domesticated 14-month-old broodstock of the giant tiger shrimp (*Penaeus monodon*). Total proteins extracted from testes of wild (group A, GSI = 1.08 ± 0.18%, sperm sac/testis = 0.26 ± 0.06,) and domesticated broodstock (group B, GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 0.01 and group C, GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02) were further analyzed by 2-DE. In total, 640 protein spots were characterized including 394 spots from wild broodstock (group A), 120 spots from domesticated broodstock group B and 126 spots from domesticated broodstock group C, respectively. Interesting proteins such as farnesoic acid-O-methyltransferase (FAMeT), progesterone receptor-related protein p23, receptor activating protein kinase C (RACK), 14-3-3-like protein and NADP-dependent leukotriene B4 12-hydroxydehydrogenase (LTB4DH) were identified.

In addition, a new proteomic analysis based on one-dimensional gel electrophoresis (1-DE) of total proteins in testes of wild (group A; GSI 0.66 ± 0.18%, sperm sac/testis = 0.51 ± 0.09 and group B; GSI = 0.68 ± 0.09%, sperm sac/testis = 0.49 ± 0.66), and domesticated 14-month-old (group C; GSI = 0.37 ± 0.03%, sperm sac/testis = 0.5 ± 0.02) and 18-month-old (group D; GSI = 0.37 ± 0.01%, sperm sac/testis = 0.44 ± 0.01) broodstock of *P. monodon* was also carried out. Approximately 50 proteins that showed large differential (up-regulation and down-regulation) expression profiles among sample groups were further annotated. In total, 345 differentially expressed proteins were identified. Interestingly, 1 (0.29%) and 18 (5.22%) proteins were found in only group A and in both groups of wild broodstock, respectively whereas 231 (66.96%) proteins were commonly found in all groups of samples. Reproduction-related proteins such as vasa-like protein, Ran GTPase activating protein 1 and seven transmembrane helix receptor were identified.

The full length cDNA of *ubiquitin specific peptidase 14* (ORF of 1524 bp corresponded to a polypeptide of 507 amino acids), *ubiquitin carboxyl-terminal hydrolase 5* (ORF of 2442 bp corresponded to a polypeptide of 813 amino acids), *Cdk17* (ORF of 1470 bp corresponded to a polypeptide of 489 amino acids) and *proteasome alpha subunit* (ORF of 765 bp corresponded to a polypeptide of 254 amino acids) of *P. monodon* was successfully identified and reported for the first time in this species. Tissues distribution analysis was examined. These genes were constitutively expressed in all examined tissues of *P. monodon* broodstock.

Quantitative real-time PCR indicated that the expression levels of testicular *serine/threonine-protein kinase 23* and *ubiquitin carboxyl-terminal hydrolase 14* between juveniles and domesticated and wild broodstock of male *P. monodon* were not significantly different ($P > 0.05$). In contrast, the expression levels of *proteasome alpha subunit* and *proteasome delta* in testes of 10- and 14-month-old shrimp were not significantly different from those of wild broodstock ($P < 0.05$) but significantly greater than those of 6-month-old juveniles and 18-month-old broodstock ($P > 0.05$). In addition, the expression levels of *26S proteasome regulatory subunit S3* in testes of domesticated 14-month-old broodstock and wild broodtock were not different ($P > 0.05$) but its expression level in 6-month-old juveniles and domesticated 10- and 18-month-old broodstock were significantly lower than that of 14-month-old shrimp ($P < 0.05$). Taken together, the expression profiles of *proteasome alpha subunit*, *proteasome delta* and *26S proteasome regulatory subunit S3* indicated that domesticated male *P. monodon* possibly reached the initial maturation period at 10 months old, attained the maximal maturation peak at 14 months old and reduced the reproductive maturation at 18 months old.

Field of Study :.....Biotechnology.....

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Student's Signature....Sasithon Petkon....

Advisor's Signature .....

Co-Advisor's Signature.....

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LIST OF ABBREVIATIONS

bp	base pair
°C	degree Celcius
DEPC	diethylpyrocarbonate
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DNA	deoxyribonucleic acid
DTT	dithiothreitol
HCl	hydrochloric acid
IAA	iodoacetamide
IPTG	isopropyl-thiogalactoside
Kb	kilobase
M	molar
$MgCl_2$	magnesium chloride
mg	Milligram
ml	Millilitre
mM	Millimolar
ng	Nanogram
OD	optical density

PCR	polymerase chain reaction
RNA	ribonucleic acid
RNase A	ribonuclease A
rpm	revolution per minute
RACE	rapid amplification of cDNA ends
RT	reverse transcription
SDS	sodium dodecyl sulfate
Tris	tris (hydroxyl methyl) aminomethane
µg	microgram
µl	microlitre
µM	micromolar
UV	ultraviolet

CHAPTER I

INTRODUCTION

1.1 Background information and objectives of this thesis

Farming of the giant tiger shrimp (*Penaus monodon*) in Thailand relies almost entirely on wild-caught broodstock for supply of juveniles because of poor reproductive maturation of cultured *P. monodon* (Withyachumnarnkul et al., 1998; Preechaphol et al., 2007). As a result, breeding of pond-reared *P. monodon* is extremely difficult and rarely produced enough quality of larvae required by the industry.

The high demand on wild female broodstock leads to overexploitation of the natural populations of *P. monodon* in Thai waters (Klinbunga et al., 1999; Khamnamtong et al., 2005). The lack of high quality wild and/or domesticated broodstock of *P. monodon* has caused a significant decrease in its farmed production since the last several years (Limsuwan, 2004). Reduced degrees of reproductive maturation in captive *P. monodon* broodstock have limited the potential of genetic improvement resulted in remarkably slow domestication and selective breeding programs of *P. monodon* in Thailand (Withyachumnarnkul et al., 1998; Clifford and Preston, 2006; Preechaphol et al., 2007).

Progress in genetic and biotechnology researches in penaeid shrimps have been slow because a lack of knowledge on fundamental aspects of their endocrinology and reproductive biology (Benzie, 1998). The domestication and selective breeding programs of penaeid shrimp would provide a more reliable supply of seed stock and the improvement of their production efficiency (Makinouchi and Hirata, 1995; Clifford and Preston, 2006; Coman *et al*., 2006). The use of selectively bred stocks having improved culture performance, disease resistance and/or other commercially desired traits rather than the reliance on wild-caught stocks is a major determinant of sustainability of the shrimp industry (Clifford and Preston, 2006).

Practically, breeding of *P. monodon* using spermatozoa of captive males yields low quality offspring. The use of spermatozoa from wild males with either wild or pond-reared females has resolved that problem successfully (B. Withyachumnarnkul, personal communication).

Baseline information related to testicular development and sperm quality in penaeid shrimp is rather limited. An initial step towards understanding molecular mechanisms of testicular and spermatozoa development in *P. monodon* is to identify and characterize differentially expressed genes and protein in various stages of testicular development of this economically important species.

Proteomic technique is a powerful and widely used method for analysis of protein mapping and differential expression of interesting proteins in various cells and tissues of organisms. Proteomics provide the basic information on protein expression profiles and post-translational modification of interesting proteins. Molecular mechanisms and expression patterns of proteins controlling testicular development of *P. monodon* could be carried out. In addition, isolation, characterization and expression analysis of genes that encode proteins related with testicular and/or sperm development of *P. monodon* provide the basic information allowing better understanding of the reproductive maturation of male *P. monodon* in captivity.

1.2 Objective of this thesis

The objectives of this thesis are determination of protein profiles in testes of wild and domesticated *P. monodon* broodstock by proteomic approaches. The full length cDNA of genes functionally related with testicular development identified by this study and those previously characterized by EST analysis were further characterized. Expression analysis of several reproduction-related genes in testes of wild and different ages of domesticated broodstock was also examined.

1.3 General introduction

1.3.1 Taxonomy of *P. monodon*

The giant tiger shrimp is taxonomically classified as a member of Phylum Arthropoda; Subphylum Crustacea; Class Malacostraca; Subclass Eumalacostraca; Order Decapoda; Suborder Natantia; Infraorder Penaeidea; Superfamily Penaeoidea; Family Penaeidae, Rafinesque, 1985; Genus Penaeus, Fabricius, 1798 and Subgenus *Penaeus*. The scientific name of shrimp is *Penaeus monodon* (Fabricius, 1798) where the English common name is giant tiger shrimp or black tiger prawn (Bailey-Brook and Moss, 1992).

1.3.2 Farming of *P. monodon* in Thailand

The giant tiger shrimp, *P. monodon* has dominated production of farmed shrimp along with the Pacific white shrimp (*Litopenaeus vannamei*) and is one of the most economically important penaeid species in South East Asia. Farming of *P.* monodon has achieved a considerable economic and social importance, constituting a significant source of income and employment in this region.

In Thailand, *P. monodon* has been intensively cultured for more than two decades. Farming activity of *P. monodon* in Thailand has rapidly increased reflecting a large annual production. The reasons for this are supported by several factors including the appropriate farming areas without serious disturbing from typhoons or cyclone, small variable of seawater during seasons, and ideal soils for pond construction. Culture of *P. monodon* increases national revenue, therefore *P. monodon* is an economically important species in Thailand.

Marine shrimp farms and hatcheries are located along the coastal areas of Thailand where Nakorn Sri Thammarat and Surat Thani located in peninsular Thailand are the major parts of shrimp cultivation. In addition, Chanthaburi (eastern Thailand), Samut Sakhon and Samut Songkhran (central region) also significantly contribute on the country production. The intensive farming system has resulted in consistent production of marine shrimp of Thailand. Thailand has been regarded as the leading shrimp producer of cultivated shrimp for over a decade (Table 1.1).

Farming of *P. monodon* in Thailand relies almost entirely on wild-caught broodstock for supply of juveniles because reproductive maturation of cultured *P. monodon* female is extremely low. As a result, breeding of pond-reared *P. monodon* is extremely difficult and rarely produced enough quality of larvae required by the industry. The high demand on wild female broodstock leads to overexploitation of the natural populations of *P. monodon in* Thai waters (Klinbunga *et al*., 1999).

The production of *P. monodon* is largely constrained by the current dependency on wild-caught broodstock which varies in both quality and quantity. Recently, the farming of *P. monodon* in the region has significantly declined As a result, *L. vannamei* has been introduced to Thailand as an alternative cultured species and become the main culture species at present (Table 1.2).

Table 1.1 Total shrimp production (metric tons) from the aquaculture sector during 2000 - 2005 in Southeast Asia

Country	2000	2001	2002	2003	2004	2005
Thailand	290,000	280,000	250,000	350,000	360,000	360,000
Indonesia	110,000	90,000	102,000	168,000	180,000	230,000
China	200,000	300,000	280,000	400,000	350,000	280,000
India	85,000	80,000	125,000	100,250	100,000	100,000
Vietnam	75,000	95,000	85,000	110,000	160,000	115,000
Malaysia	17,000	20,000	24,000	280,000	280,000	320,000
Philippines	30,000	20,000	30,000	30,000	35,000	35,000
Total	807,000	885,000	896,000	1,186,250	1,213,000	1,152,000

(Source: World shrimp farming, 2004)

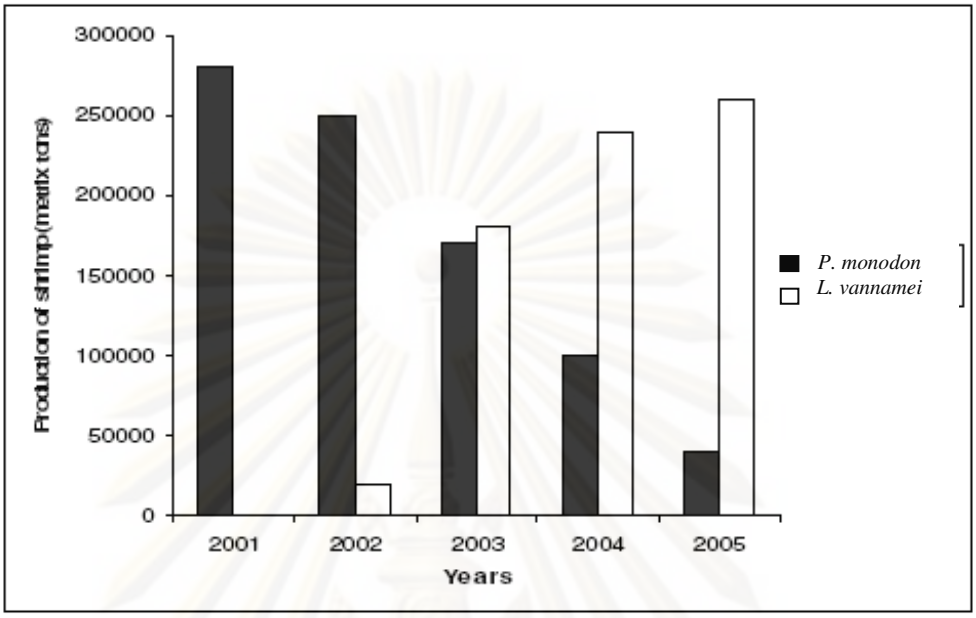

Figure. 1.1 A diagram of production of *P. monodon* and *L. vannamei* during 2001-2006 in Thailand

Nevertheless, *P. monodon* is a local species. The domestication and selective breeding programs of *P. monodon* would provide a more reliable supply of seed stock and the improvement of its production efficiency (Makinouchi and Hirata, 1995; Clifford and Preston, 2006; Coman *et al*., 2006). The use of selectively bred stocks having improved culture performance; disease resistance and/or other commercially desired traits (e.g. fast growth) rather than the reliance on wild-caught stocks is a major determinant of sustainability of the shrimp industry (Benzie, 1998; Clifford and Preston, 2006).

Moreover, the price of *L. vannamei* is quite low and broodstock used relies almost entirely on genetic improved stocks brought from different sources. The labor costs in Thailand are higher than other countries (e.g. Vietnam and China) preventing the advantage of competition for the world market. In contrast, the market of premium-sized *P. monodon* is still open for Thailand because *L. vannamei* is not suitable for that market. Accordingly, *P. monodon* culture is currently promoted for increasing the production of this species.

Table 1.2 Export of the giant tiger shrimp from Thailand during 2002-2007

Country	2002		2003		2004		2005		2006		2007	
	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)
USA	97681.81	36,011.41	89115.28	29,032.87	58365.2	17,206.75	29116.62	17,206.75	34537.23	8,847.42	7979.91	1,909.64
Japan	16644.6	13,813.33	33235.52	11,916.87	27977.27	9,586.59	20182.85	9,586.59	15,709.39	3,832.31	3711.32	1,067.25
Canada	6455.76	3,890.48	11216.47	3,412.09	6490.03	2,072.25	3249.37	2,072.25	2798.61	744.95	1762.16	462.68
Singapore	5251.66	3,138.86	3317.14	1,258.13	3383.18	537.88	1933.5	537.88	1580.11	236.31	401.47	63.53
Taiwan	4917.65	1,276.86	3051.77	799.44	2964.62	564.58	1673.65	564.58	607.7	170.12	692.69	194.78
Australia	4481.25	1,326.06	4817.5	1,252.31	2418.19	1,042.02	2097.76	1,042.02	1418.36	445.05	658.54	225.13
Hong Kong	1365.12	533.26	1437.54	340.42	1396.98	409.93	1026.84	409.93	921.88	256.91	1569	365.91
Chaina	1649.23	352.68	992.91	214.54	833.1	162.66	1003	162.66	710.7	85.65	1629.74	235.57
U. Kingdom	661.07	210.81	184.23	64.11	505.76	181.63	161.79	181.63	241.91	70.54	242.4	73.46
Total	180,615.81	63,822.73	160,986.48	51,524.10	118,343.12	16,629.05	69,168.96	16,629.05	64,565.41	16,178.85	23,933.1	5,922.11

Source: http://www.fisheries.go.th/foreign/doc/excel/export_backtiger.xls

1.4 The reproductive organs and hormonal control of marine shrimp

The male reproductive system includes paired testes, paired vas deferens, and a petasma (Fig. 1.2 B). Mating of *P. monodon* occurs at night after the female molts. Sperm is deposited into a special structure called the thelycum on the underside of the female's thorax (Fig. 1.2 C). A single female usually produces 250,000-800,000 eggs, which are freely released into the water and hatch within 18 hours into nauplii larvae. The external morphology of *P. monodon* and sex characteristics of male (petasma) and female (thelycum) are illustrated in Figure 1.2 A.

Biological and physiological processes (growth, reproduction, body color, and metabolism etc.) in shrimp are hormonal controlled (Figure 1.3). Knowledge from shrimp endocrinology is necessary to develop the hormonal manipulation techniques

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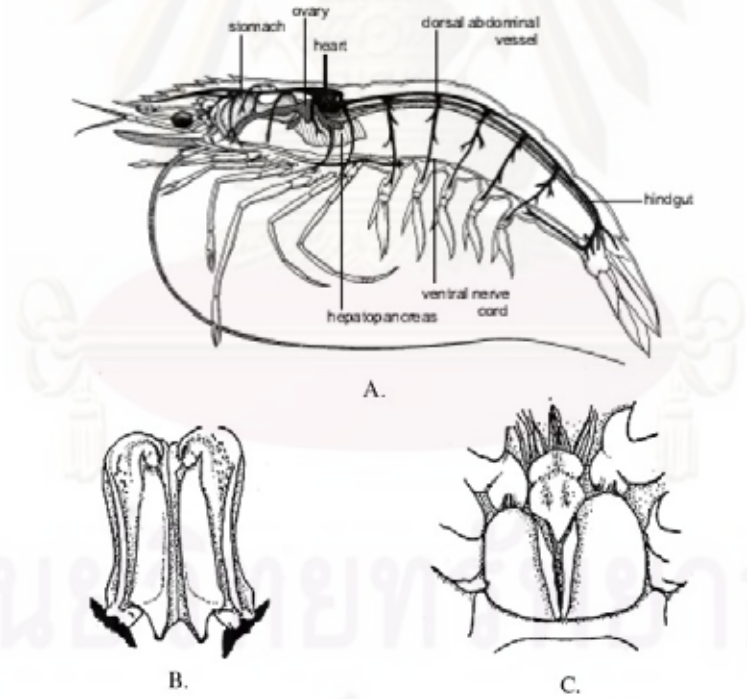


Figure. 1.2 External morphology of *P. monodon* (A). Sexes of juveniles and broodstok of penaeid shrimp can be externally differentiated by petasma of male (B) and thelycum of female (C).

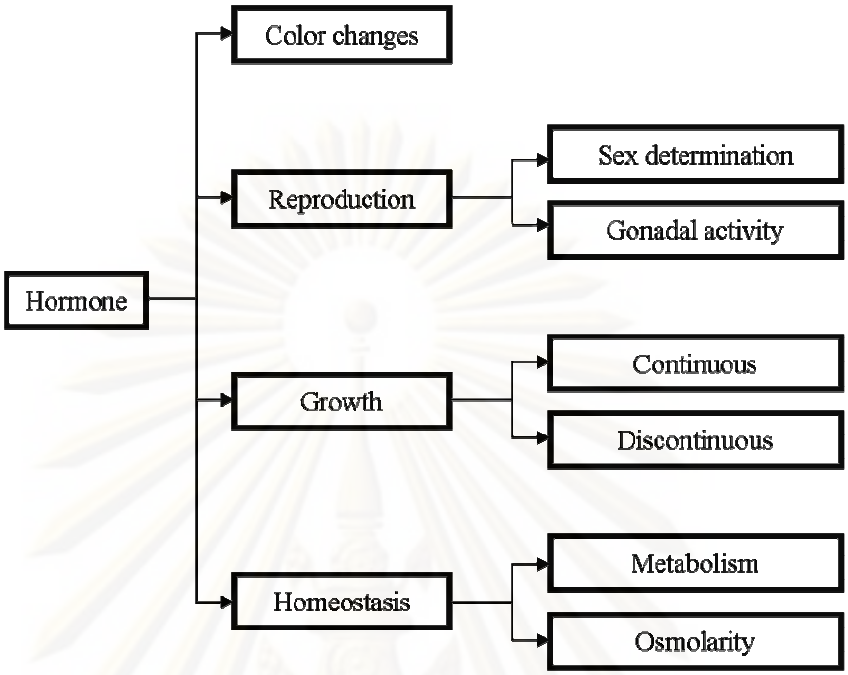


Figure 1.3 Diagram illustrating the hormonal controls of physiological processes of penaeid shrimp.

in shrimp. Eyestalk hormones play the important role for regulating several physiological mechanisms and unilateral eyestalk ablation is practically used for induction of ovarian development but this technique does not have the potential effects on testicular development of *P. monodon*. Therefore, the molecular mechanisms controlling testicular and ovarian maturation may be different.

1.5 Spermatogenesis

Spermatogenesis is a complex cell differentiation process required a coordinated series of both mitosis and meiosis cycle events (Abe, 1987) and consists of a series of complex cellular events, in which different genes express to ensure the proper development of spermatozoa. The process of spermatogenesis follows an endocrine-regulated developmental program that features the transformation of an undifferentiated diploid stem cell into highly differentiated haploid spermatozoa.

In mammals, spermatogenesis is composed of three stages; the mitotic

proliferation of spermatogonia, meiotic division of spermatocytes, and morphogenetic processes converting haploid spermatids to spermatozoa (Abe, 1987). Spermiation and sperm maturation occur during the final stage of spermatogenesis and are critical step for successful fertilization (Callard, 1991; Zirkin, 1993). The mitotic proliferation of spermatogonia includes the germinal stem cells and other mitotic germinal cells produced from the stem cells (Grimes, 2004) and starts with the self-renewal and differentiation of a small population of spermatogonial stem cell. Spermatogonial stem cells are found in the basal part of the seminiferous epithelium, in contact with the basement membrane. They are also in close association with the nursing Sertoli cells, which produce the growth factors necessary to induce self-renewal and differentiation (Braydich-Stolle *et al*., 2007).

For the second stage, meiotic division of spermatocytes, DNA replication does not occur in spermatocytes but DNA repair is critical during this time period. Many unique genes are involved in the process of genetic recombination for example unique genes encode SCP1 and COR1 proteins are components of the synaptonemal complex, protein involved in recombination and DNA repair, and the *Dmc1* gene are all expressed in spermatocytes (Grim, 2004).

In addition, a targeted mutation of 70-kDa heat-shock gene Hsp70-2, which is expressed in the meiotic phase of spermatocytes in mice, leads to infertility. Development is arrested in late pachytene spermatocytes at the G2/M phase of the meiotic cell cycle. Hsp70-2 may be molecular chaperones required for Cdc2 activation that may facilitate dimerization of Cdc2 with cyclinB1 to become and active kinase in male germ cells (Eddy, 1999).

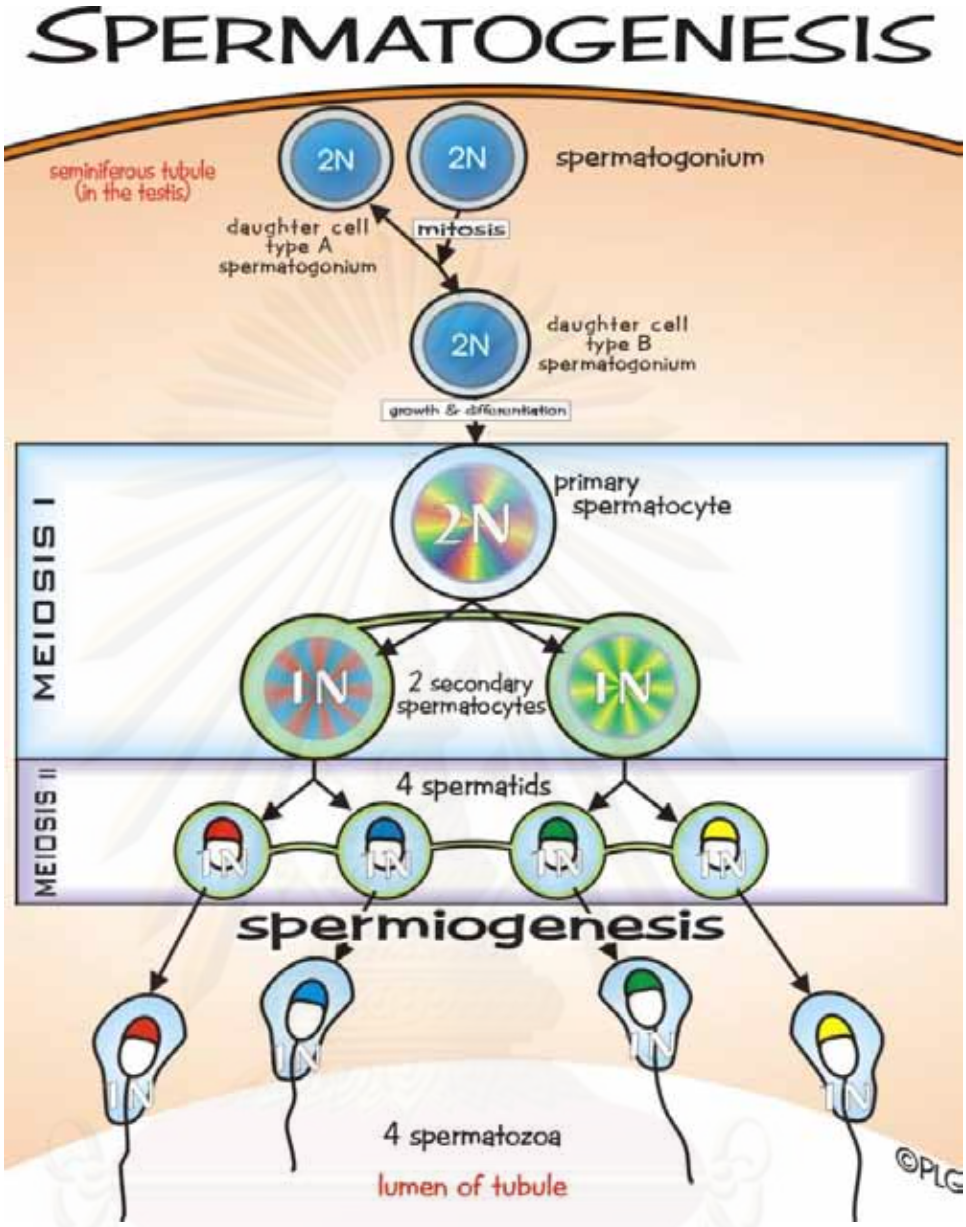


Figure 1.4 General diagram of spermatogenesis (from: science.tjc.edu/images/reproduct.tomy.htm)

The third stage, called spermiogenesis, is morphogenetic processes converting haploid spermatids to mature spermatozoa. Many molecular events occur in spermatids that are required for completion of spermatogenesis. Significant progress has been made in understanding the unique chromatin remodeling and regulation of post-meiotic transcription in male germ cells that occurs during spermiogenesis (Sassone-Corsi, 2002). There is greatly increased transcriptional activity giving rise to several indispensable post-meiotic proteins in the early spermatids. For example, testis-specific isoforms of TATA-binding protein (TBP) are typically found (Sassone-Corsi, 2002). cAMP-responsive elements (CREs), members of the CREB family of transcription factors (Sassone-Corsi, 1998) are poorly expressed in testis, but another CREB family member, CREM, is present at high levels.

1.6 Molecular techniques used in this thesis

1.6.1 Two-dimensional electrophoresis

Two-dimensional electrophoresis is a powerful and widely used method for the analysis of complex protein mixture extracted from cells, tissues or other biological samples. This technique sorts proteins according to two independent properties in two steps. Initially, samples are prepared by extraction of proteins in the appropriate buffer. The extracted proteins are then electrophoretically analyzed.

The first dimension step, isoelectric focusing (IEF), separates proteins according to their isoelectric points (p*I*). Proteins are amphoteric molecules; they carry either positive, negative or zero net charges, depending on the pH of their surroundings. The net charge of a protein is the sum of all the negative and positive charges of its amino acid side chain and amino and carboxyl-termini. The isoelectric point (p*I*) is the specific pH at which the net charge of the protein is zero. Proteins are positively charged at pH values below their p*I* and negatively charged at pH values above their p*I*.

The IEF step is the most critical step for 2-DE process. During IEF, protein mixtures must be solubilized in denaturing buffer without non-ionic detergents, usually in chaotrophs [high concentrate urea solution (8M urea or 7M urea with 2M thiourea)] together with surfactant (CHAPS) and reducing agents (DTT). To obtain

high quality separation, samples protein should be optimized to select a suitable range of isoelectric focusing pH gradients due to different types of interesting proteins.

The second dimension step, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), separates proteins according to their molecular weights. Each spot on the resulting two-dimensional array corresponds to a single (or mixed) protein in the sample. Thousands of different proteins can thus be separated, and information such as the protein p*I*, the apparent molecular weight, and the amount of each protein is obtained. The resolution of the secondary separation can be optimized be varying the percentage of crosslink of the acrylamide gel.

After electrophoresis, the separated proteins must be visualized in the gel. Most commonly, this is achieved with dyes that firmly bind protein. Individual dyes differ in sensitivity and the ability to stain all types of proteins equally. The most frequently use dye is Coomassie Blue R-250 (with a direction limit of about 1 μg of a protein). Alternatively, Coomassie Blue G-250, Amido black, and Nigrosine are also used.

Silver staining is more sensitive than Coomassie Blue staining for about 10 - 20 fold. Silver staining leads to a non-stoichiometric binding of silver ions to proteins. After reduction, these complexes become visible as black to brownish bands. Unfortunately, silver stains are inconsistent as some proteins are hardly stained by silver ions. Therefore, quantity of stained proteins is not proportionally indicated from intensity of the protein spots. Fluorescent staining has been recently developed as an alternative choice for high sensitivity of staining. Dyes including SyproRuby™, deep Purple™ and 5-hexadecanoylamino-fluorescein are commercially available. However, the fluorescent staining is more expensive than conventional Coomassie Blue and silver staining and requires a specific gel documentation for visualization of electrophoresed proteins.

1.6.2 Mass spectrometry

Mass spectrometry is a highly sensitive technique of instrumental analysis of molecules invented about 90 years ago. In the 1950s, mass spectrometry expanded into organic chemistry. Today, a wide range of mass spectrometry types that are specialized for the analysis of elements, small gaseous molecules, or biomolecules

and biopolymer, exists. Protein identification by this analysis used proteomically digested protein to give higher accuracy of identification than the intact proteins. Proteolysis is achieved using common enzymes such as trypsin prior to MS analysis. This enzyme hydrolyzes peptide bonds on the C terminal side of lysine (Lys) and arginine (Arg) residues, except when they are immediately followed by proline (Pro). Other enzymes such as pepsin, proteinase K and even chemical digestion using reagent such as cyanogenbromide (CNBr) can also be used for the protein digestion However, the use of CNBr yields large peptide fragments that may not useful for peptide sequencing by MS.

Mass spectrometers are made up of three functional units: an ion source, a mass analyzer, and a detector. For mass spectrometric analyses, free gaseous ions are generated from the sample in the ion source and then focused into and ion beam in vacuum. The mass analyzer separates ions in this beam according to their mass/charge(m/z)-ratio; these ions are then registered by detector. Individual measurements are plotted in a mass spectrum with m/z (x-axis) and intensity (y-axis) as show in (Fig 1.5).

Two techniques of mass spectrometry have established in biomolecular analysis; matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI).

Electrospray ionization involves spraying the analyte solution from a microcapillary that carries a high (negative or positive) potential in reference to the mass spectrometer. When the electrostatic force of the applied current exceeds the surface tension of the analyte solution, a *Taylor cone* forms at the tip of the microcapillary. Highly charged droplets form and solvent evaporation disintegrates them further to a fine spray. This analyte spray is then sucked into the evacuated mass. analyzer through a microorifice. In the interface area, the droplets are dried and ion formation occurs. The working schematic of an ESI ion source is show in (Fig. 1.6).

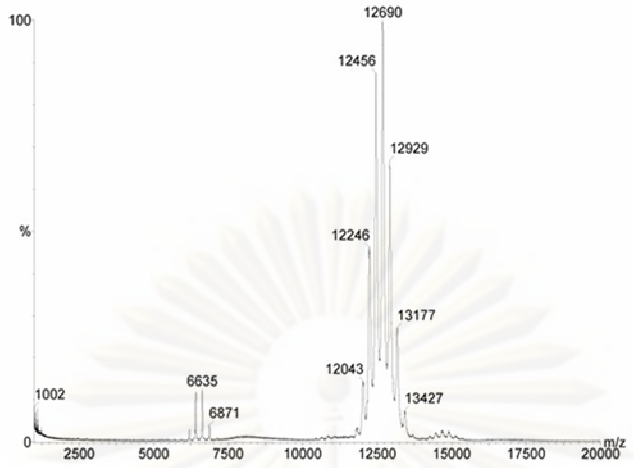


Figure 1.5 Partial ESI mass spectra with two signals from doubly peptides. The registered ion m/z-values (mass-to-charge ratio) are plotted on the x-axis, their intensity on y-axis. www.waters.com/…/LCT_Premier_detail_3.jpg .

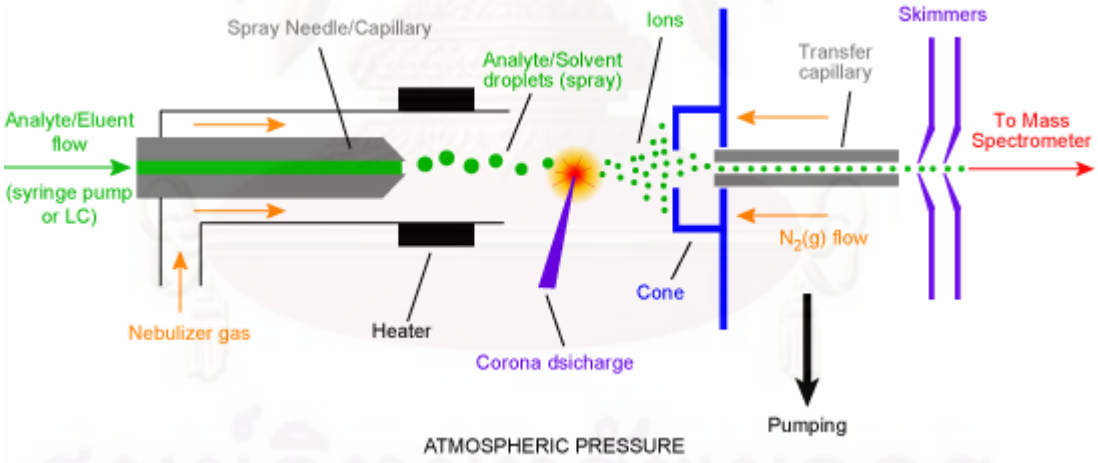


Figure 1.6 Schematic view of an electrospray ion source. Analyte solution is sprayed at atmospheric pressure, droplets enter the evacuated analyzer area through a microorifice and anion beam is formed (www.bris.ac.uk/nerclsmsf/techniques/hplcms.html).

1.6.3 PCR

The introduction of the polymerase chain reaction (PCR) by Mullis *et al*. (1987) has opened a new approach for molecular genetic studies. This method is technique for enzymatically replicating DNA without using a living organism, such as *E. coli* or yeast and is a method using specific DNA sequences by the two oligonucleotide primers, usually 18-25 nucleotides in length. Million copies of the target DNA sequence can be synthesized from the low a mount of starting the DNA template within a few hours.

The PCR components are composed of DNA template, a pair of primers for the target sequence, dNTPs (dATP, dCTP, dGTP and dTTP), PCR buffer and heat-stable DNA polymerase (usually *Taq* polymerase). The amplification reaction typically consists of three steps; denaturation of double stranded DNA at high temperature, annealing to allow primers to form hybrid molecules at thr optimal temperature, and extension of the annealed primers by heat-stable DNA polymerase. The cycles are repeated for 30-40 times (Figure 1.7). The amplification product is determined by agarose or polyacrylamide gel electrophoresis.

1.6.4 Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR is a comparable method of conventional PCR but the first strand cDNA template rather than genomic DNA was used as the template in the amplification reaction (Figure 1.8). It is a direct method for examination of gene expression of known sequence transcripts in the target species. The template for RT-PCR can be the first stranded cDNA synthesized from total RNA or poly A^+ RNA. Reverse transcription of total RNA can be performed with oligo(dT) or random primers using a reverse transcriptase The product is then subjected to the second strand synthesis using a gene-specific forward primer.

RT-PCR can also be used to identify homologues of interesting genes by using degenerate primers and/or conserved gene-specific primers from the original species and the first strand cDNA of the interesting species is used as the template. The amplified product is further characterized by cloning and sequencing.

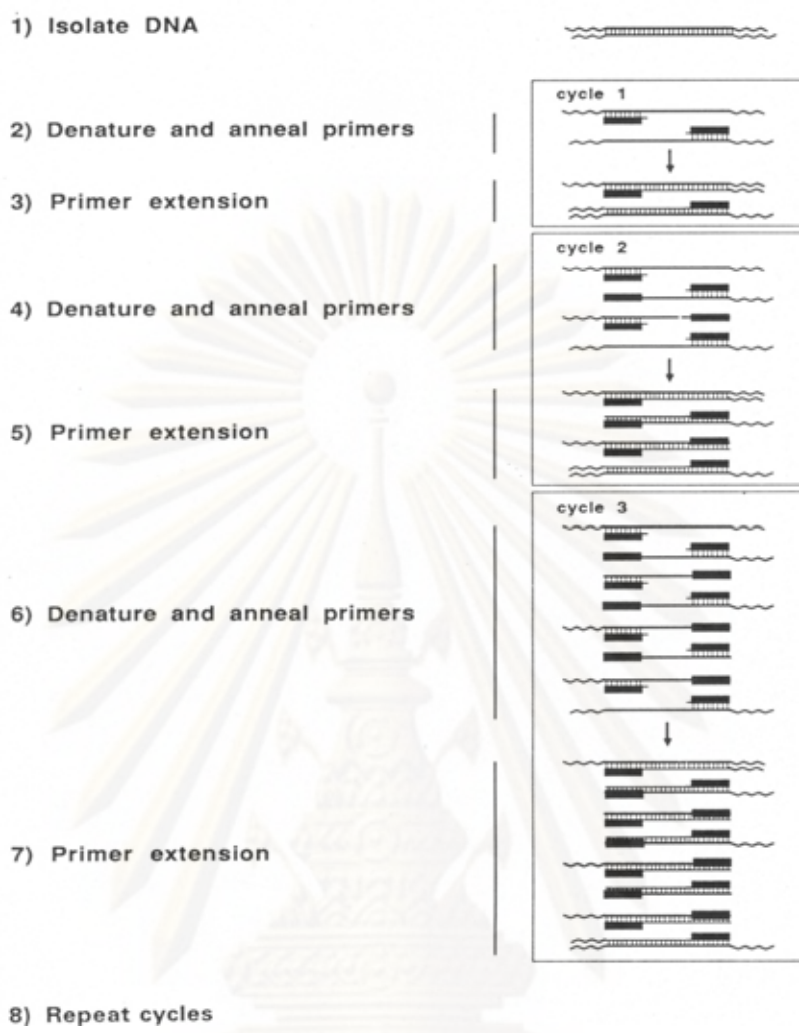


Figure 1.7 General illustration of the polymerase chain reaction (PCR) for amplification of the target DNA.

Semi-quantitative RT-PCR is a relatively quantitative approach where the target genes and the internal control (e.g. a housekeeping gene) were separately or simultaneously amplified using the same template. The internal control (such as *β-actin*; *elongation factor*, *EF-1α* or *G3PDH*) is used under the assumption that those coding genes are transcribed constantly and independently from the extracellular environment stimuli and that their transcripts are reverse transcribed with the same efficiency as the product of interesting transcript.

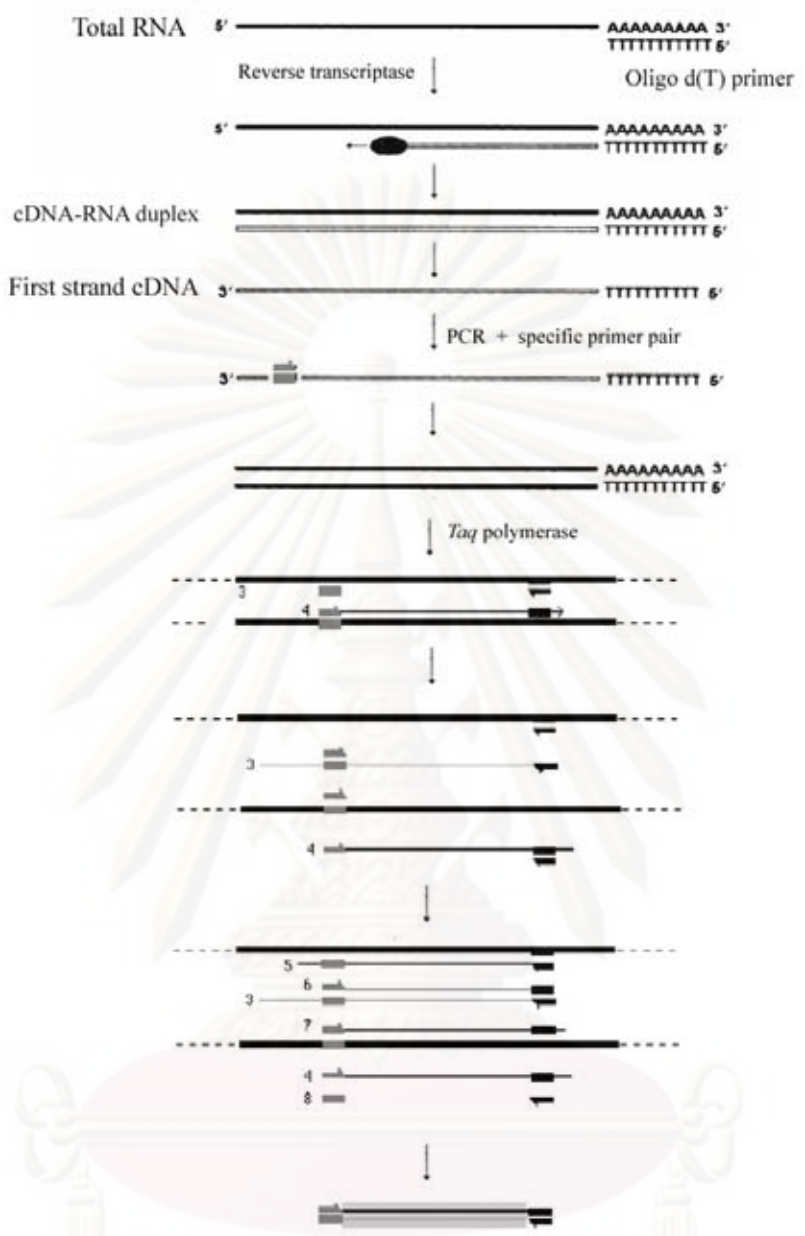
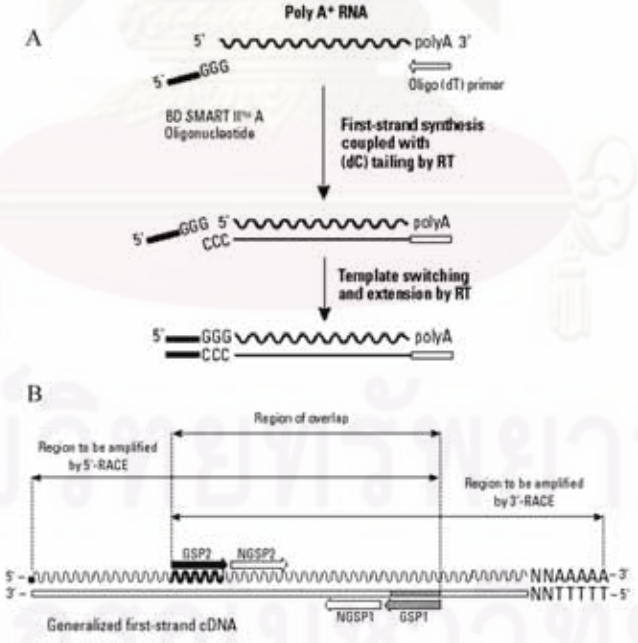


Figure 1.8 Overall concepts of RT-PCR. During the first strand cDNA synthesis, an oligo d(T) (or random primers) primer anneals and extends from sites present within mRNA. The second strand cDNA synthesis primed by the 18 – 25 base specific primer proceeds during a single round of DNA synthesis catalyzed by thermostable DNA polymerase (e.g. *Taq* polymerase)

1.6.5 Rapid amplification of cDNA ends-polymerase chain reaction (RACE-PCR)

RACE-PCR is the common approach used for isolation of the full length of characterized cDNA. Using SMART (Switching Mechanism At 5′ end of RNA Transcript) technology, terminal transferase activity of Powerscript Reverse Transcriptase (RT) adds 3 - 5 nucleotides (predominantly dC) to the 3′ end of the first-strand cDNA. This activity is harnessed by the SMART oligonucleotides whose terminal stretch of dG can anneal to the dC-rich cDNA tail and serve as an extended template for reverse transcriptase. A complete cDNA copy of original mRNA is synthesized with the additional SMART sequence at the end (Fig. 1.9). The first strand cDNA of 5′ and 3′ RACE is synthesized using a modified oligo (dT) primers and serve as the template for RACE PCR reactions. Gene specific primers (GSPs) are designed from interested gene for 5′- RACE PCR (antisense primer) and 3′-RACE PCR (sense primer) and used with the universal primer (UPM) that recognize the SMART sequence. RACE products are characterized. Finally, the full length cDNA is constructed.



(SMART^{TM} RACE cDNA Amplification Kit User Manual, Clontech)

Figure 1.9 Overview of the SMART^{TM} RACE cDNA Amplification Kit.

A. Mechanism of SMART cDNA synthesis. First strand synthesis is primed using a modified oligo (dT) primer. After reverse transcriptase reaches the end of the mRNA template, it added several dC residues. The SMART II A Oligonucleotide annels to the tail of the cDNA and serves as an extended template for PowerScript RT.
B. Relationships of gene-specific primers to the cDNA template. This diagram shows a generalized first strand cDNA template.

1.6.6 Real-time PCR

Real-time PCR is a kinetic approach based on the polymerase chain reaction, which is used to amplify and simultaneously quantify a target DNA molecule. It enables both detection and quantification (as absolute number of copies or relative amount when the expression level of the target gene is normalized by that of the reference gene) of a specific sequence in the sample.

The real-time PCR procedure follows the general principle of PCR. Its key feature is that the amplification DNA is quantify as it accumulates in the reaction in the real time situation after each amplification cycle. Two common methods of quantification are the use of fluorescent dyes that intercalate with double-stranded DNA such as SYBR green and modified DNA oligonucleotide probes that are fluorescent when hybridized with a complementary DNA.

The general principle of SYBR green polymerase chain reaction is composed of the first step, denaturation: at the beginning of amplification, the unbound dye molecules are weakly fluorescent, the second step, annealing: after annealing of the primer, a few dye molecules bind to the double strand. The last step, extension: during elongation, more dye molecules bind to the newly synthesized DNA. Fluorescence measurement at the end of the elongation step of every PCR cycle is performed to monitor the increasing the amount of quantified DNA (Fig. 1.10).

Real-time PCR in the laboratory can be applied to numerous applications. It is common use for both diagnostic and research applications. Diagnostic real-time PCR is applied to rapidly detect the presence of genes involved in infection diseases, cancer and genetic abnormalities. In the research setting, real-time PCR is mainly use to provide highly sensitive quantitative measurement of gene transcription. The

technology is commonly used in determining expression levels of a particular gene changes over time.

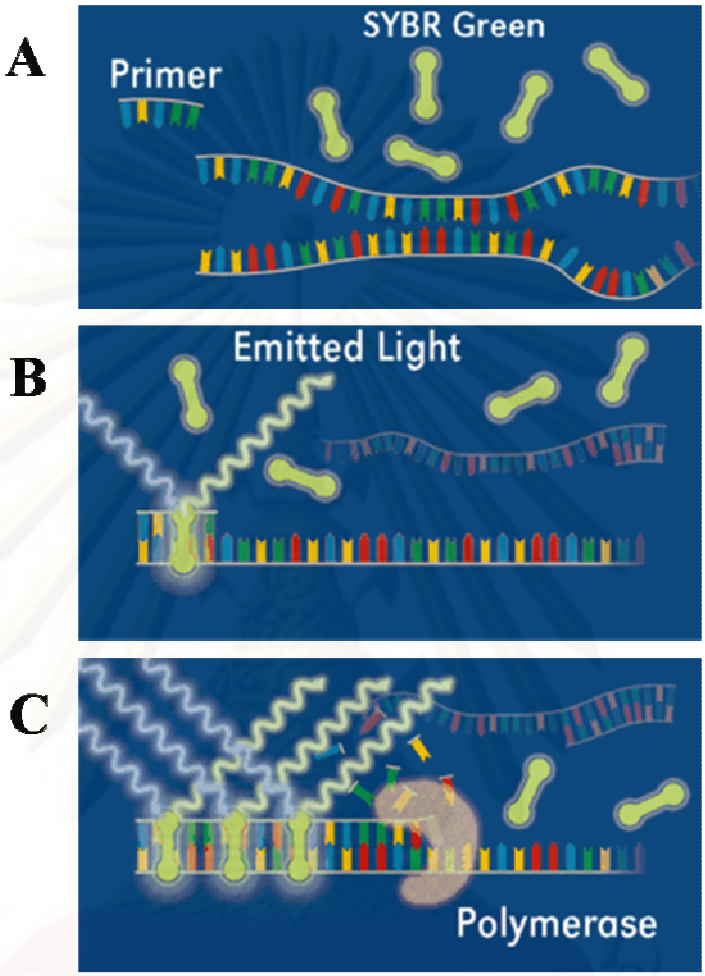


Figure 1.10 An overconcept of the Real-time PCR procedure (**www.thaiscience.com/lab_vol/p23/Real-time PCR.asp**).

1.7 Isolation and characterization of genes functionally involved with testicular development and spermatogenesis in various species

The Doublesex Male abnormal-3 Related Transcription factor-1 (DMRT1) gene encodes a protein containing the DNA-binding motif called the DM domain, involved in the sexual development of various species. Klinbunga *et al* (2009) identified and characterized a *DMRT1* homolog in the tropical abalone (*Haliotis*

asinina). The full length cDNA of *Ha-DMRT1* (1,740 bp with an ORF of 732 bp corresponding to a putative polypeptide of 243 amino acids) and its DM domain-less variant (*Ha-DMRT1-like*, 1,430 bp with an ORF of 312 bp, 103 amino acids) were successfully isolated and reported for the first time in molluscs. *Ha-DMRT1* was specifically expressed in the testes of adult *H. asinina* (N = 16) but not in whole juveniles (2, 3, 5 months old, N = 6 for each group) and ovaries (N = 16), and pooled hemocytes (from 50 individuals) of adults. Tissue distribution analysis further revealed testis-specific expression of *Ha-DMRT1*. Semiquantitative RT-PCR illustrated that the relative expression level of *Ha-DMRT1* in developed testes (stages II, III, and IV) was significantly greater than that in undeveloped testes (stage I) of abalone broodstock ($P < 0.05$).

Subsequently, the genes *Tektin A1* and *axonemal protein 66.0* were also successfully isolated and characterized in *H. asinina*. The full-length cDNAs of *Ha-TekA1* and *Ha-Axp66.0* were 2166 and 2038 bp long, with ORFs of 1350 and 1683 bp, respectively. Both *Ha-TekA1* and *Ha-Axp66.0* were expressed in the testes but not in the ovaries or hemocytes of *H. asinina* adults. In addition, *Ha-Axp66.0* was not expressed in *H. asinina* juveniles (2, 3, and 5 months old). A tissue expression analysis showed *Ha-Axp66.0* to be expressed specifically in the testes, whereas *Ha-TekA1* was expressed abundantly in the testes but weakly in the foot, gill, digestive gland, left hypobranchial gland, and mantle. The relative expression levels of *Ha-TekA1* and *Ha-Axp66.0* were significantly lower in undeveloped testes (stage I) than in developed testes (stages II, III, and IV) of *H. asinina* ($P < 0.05$) (Klinbunga *et al*., 2009)

In *P. monodon*, a suppression subtractive hybridization (SSH) library between cDNAs of testes and ovaries of *P. monodon* was constructed but only 61 clones were sequenced. Almost all of the ESTs (59 clones, 96.7%) in *P. monodon* testes were unknown transcripts. Only two known transcripts representing *antilipopolysaccharide* (anti-LPS) and *serine protease HTRA3* homologues were isolated (Leelatanawit *et al*., 2004).

In addition, sex-specific (or differential) expression markers in ovaries and testes of *P. monodon* were analyzed by RAP-PCR (150 primer combinations). Twenty-one and fourteen RAP-PCR fragments specifically/differentially expressed in

ovaries and testes of *P. monodon* were successfully cloned and sequenced. Expression patterns of 25 transcripts were tested against the first stranded cDNA of ovaries and testes of 3-month-old and broodstock-sized *P. monodon* (N = 5 and N = 7 – 10 for females and N = 4 and N = 5 – 7 for males, respectively). Five (FI-4, FI-44, FIII-4, FIII-39 and FIII-58) and two (M457-A01 and MII-51) derived RAP-PCR markers revealed female- and male-specific expression patterns in P. monodon. Surprisingly, MII-5 originally found in testes showed a higher expression level in ovaries than did testes of juvenile shrimps but but a temporal female-specific pattern in *P. monodon* adults (Khamnamtong *et al*., 2006).

Moreover, 896 clones from the testis cDNA library were sequenced. A total of 606 ESTs (67.6%) significantly matched sequences in the GenBank (*E*-value 1e–04) whereas 290 ESTs (32.4%) were newly unidentified transcripts. The full length cDNA of genes functionally involved in testicular development including *cyclophilin A*, *small ubiquitin-like modifier 1* (*SUMO-1*), *ubiquitin conjugating enzyme E2*, *dynactin subunit 5, cell division cycle 2* (*cdc2*) and *mitotic checkpoint BUB3* were discovered. In addition, *Tra-2*, a gene involving sex determination cascades, was successfully characterized by RACE-PCR. Expression analysis indicated that a homologue of low molecular weight neurofilament protein XNF-L (termed *P. monodon testis-specific transcript 1*, *PMTST1*; N = 8 for each sex) was only expressed in testes but not ovaries. *CYA*, *thyroid hormone receptor-associated protein complex 240 kDa component* (*Trap240*), *multiple inositol polyphosphate phosphatase 2* (*MIPP2*) and *heat shock-related 70 kDa protein 2* (*HSP70-2*), but not *SUMO-1*, *Tra-2* and *prohibitin2* were differentially expressed between ovaries and testes of P. monodon. Expression of *PmTST1* was up-regulated but that of the remaining genes in testes of *P. monodon* broodstock was down-regulated after shrimp were molted ($P < 0.05$). Significant reduction of *SUMO-1* and increment of *prohibitin2* transcripts in domesticated broodstock ($P < 0.05$) suggested that these reproductively related genes may be used as biomarkers to evaluate reduced degrees of the reproductive maturation in domesticated *P. monodon*.

Ubiquitin proteasome pathway, UPP is involved in numerous cellular processes, such as cell cycle progression (Goebl *et al*, 1988), organelle biogenesis (Spees *et al*, 2003), and transcriptional regulation (Hochstrasser *et al*, 1991).

Ubiquitination and degradation of proteasome and deubiquitination of USPs jointly maintain the appropriate intracellular levels of proteins. Therefore, UPP contributes to several control mechanisms of gametogenesis and sperm quality (Sutovsky *et al*, 2001).

In *Marsupenaeus japonicus*, *ubiquitin-conjugating enzyme E2r* (*UBE2*) was expressed at a higher level in testes than in ovaries. The expression at the stage I (GSI = 0.33 ± 0.004, $N = 5$) was significantly lower than that of the stage II (GSI = 0.45 ± 0.12, $N = 5$) but comparable to that of the stage III (GSI = 0.57 ± 0.006, $N = 5$) of testes. *UBE2* in ovaries was up-regulated since the stage III of ovaries. This suggested that *UBE2* has an important role in spermatogenesis and oogenesis of *M. japonicus* (Shen *et al*., 2008).

1.8 Proteomics studies for isolation and characterization of reproduction-related proteins in various organisms

Paz *et al*. (2006) comparatively analyzed proteomic profiles of the soluble proteins expressed at different stages of mouse testis development (8, 18 and 45 postnatal day). After comparative analysis, 44 proteins or variant forms were further identified by MALDI-TOF. Six proteins were classified as uniform expression, the protein from this group are either involved in carbohydrate metabolism or oxidoreductase activity. Nine proteins showed significant down-regulation ($P < 0.05$). These protein expression occurs mainly in the Sertoli cells/spermatogonia (8 dpn) and spermatocytes (18 dpn), becoming reduced or even abolished in postmeiotic spermatids. However, Ran GDP-binding protein, glutathione S-transferase (GST) A4, and one of the two forms of aldo-keto reductase 1B8 showed increased accumulation at 18 dpn, suggesting their relative stronger expression in meiotic spermatocytes. These up-regulated proteins were detected mainly in 45 dpn maps, and only weakly or not at all in 8 and 18 dpn protein maps. This accumulation pattern indicates a strong relationship with the presence and differentiation of round and elongation spermatids. Most of the up-regulated cytosolic proteins identified were involved in oxidoreductase processes (isocitrate dehydrogenase 1, aldo-keto reductase B8, peroxiredoxin 4, hydroxyacyl glutathione hydrolase, DJ-1 and GSTM5). Sixteen identified proteins detected in testis exhibited changes of protein levels, but they did not reach the significant level ($P < 0.05$) during the testis development.

In sturgeon aquaculture, the fish are sexed by an invasive surgical examination of the gonads. Development of a non-invasive procedure for sexing fish based on a molecular method is of special interest. Keyvanshokooh *et al*. (2008) applied a proteomics approach to analyze a differential protein expression between mature male and female gonads of the Persian sturgeon (*Acipenser persicus*). When comparing protein patterns on the 2-DE gels of the testis and ovary, 48 unique spots were distinguished in testis while only two spots were matchless in ovary. The largest group of sturgeon testis proteins (31.8%) was related to metabolism and energy production. Proteins related to translational and transcriptional regulation or DNA- and RNA-binding protein, such as aspartyl-tRNA synthetase, accounted for 20.4% of identified sturgeon testis proteins. Testicular proteins identified as chaperones, heat shock proteins and oxidative stress defense enzymes (16%) were also observed. Three protein spots identified as heat shock proteins (HSPs). The cell structure protein class (16%) was composed of cytoskeletal proteins such as tubulin and actin. The remaining 15.8% of the identified testis proteins are implicated in diverse functions such as signal transduction (6.8%), transport (6.8%), and cell division (2.2%). No ovarian and testicular proteins were directly linked to a sex-determining gene.

Recently, Talakhun (2009) used two-dimensional gel electrophoresis (2-DE) to examine the protein profiles in different ovarian stages (I, II, III and IV ovaries, respectively) of normal and eyestalk-ablated *P. monodon* broodstock. Protein spots after 2-DE were further analyzed by nanoLC-MS/MS. A total of 375 protein spots (215 spots from stage II and 160 spots from stage IV) from ovaries of normal *P. monodon* broodstock were examined. Of which, 90 (41.86%) and 102 (63.75%) spots of respective ovarian stages were significantly homologous to known proteins. The remaining 183 (125 and 58 from stages II and IV, 58.14 and 36.25%, respectively) protein spots did not match any protein and were regarded as novel uncharacterized proteins of *P. monodon*. In addition, 300 protein spots (180 and 120 spots from stages II and IV) from ovaries of eyestalk-ablated *P. monodon* broodstock were also characterized. A total of 85 (47.22%) and 41 (34.16%) proteins matched proteins in the databases, respectively. A large number of unknown protein; 95 and 79 protein spots accounting for 52.77 and 65.83%, were observed. Results clearly indicated that additional unknown proteins expressed at stage IV ovaries were induced by eyestalk ablation. The full length cDNA of *protein disulfide isomerase* (*PDI*) and *valosin*

containing protein (*VCP*) were successfully characterized. Expression of *VCP* and *PDI* were examined by quantitative real-time PCR. *VCP* was comparably expressed during ovarian development of normal *P. monodon* but was up-regulated at the final stage of ovarian development in eyestalk-ablated *P. monodon* broodstock. Considering expression levels of *PDI* in both normal and eyestalk-ablated *P. monodon* simultaneously, *PDI* was up-regulated at stage III ovaries of normal shrimp ($P < 0.05$) but its levels were not significant different during ovarian development of eyestalk-ablated broodstock ($P > 0.05$).

CHAPTER II

MATERIALS AND METHODS

2.1 Experimental animals

Specimens for proteomic studies were wild male broodstock of *P. monodon* caught alive from Andaman Sea and domesticated broodstock cultured for 14 and 18 months at the Broodstock Multiplication Center, Burapha University, Chanthaburi Campus. The gonadosomatic index (GSI, testis weight/body weight x 100) and a ratio between the sperm sac and testicular weight of each shrimp was calculated.

For RT-PCR analysis, male and female *P. monodon* juveniles (approximately 20 g body weight, 4-month-old) were purchased from a commercial farm in Chachengsao province, eastern Thailand.

For quantitative real-time PCR analysis, male *P. monodon* juveniles (N = 10, average body weight of 33 g, 6-month-old) and domesticated broodstock: 10-month-old (N = 24, average body weight of 49.91 ± 2.00 g), 14-month-old (N = 50, average body weight of 65.02 ± 1.11 g), and 18-month-old (N = 41, average body weight of 74.18 ± 8.15 g) were also collected from Burapha University.

Testes were dissected out from each shrimp and placed in the microcentrifuge tube. Other shrimp tissues, if required, were also dissected. Tissues were immediately placed in a liquid nitrogen tank, transported back to the laboratory and kept in a -80ºC freezer until needed.

2.2 Proteomic analysis of testicular proteins of *P. monodon*

2.2.1 Experimental animals

Specimens for proteomics based on two dimensional gel electrophoresis and nanoLC-MS/MS were wild male *P. monodon* broodstock (N = 3, average body weight 138.88 ±17.10, GSI =1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06 for group A) and 14-month-old domesticated broodstock which were divided in to two group

according to percent of sperm sac/weight of testis (*N* = 3 for each group with the average body weight of 61.70 ± 2.1 g, GSI = 0.37 ± 0.05% and sperm sac/testis = 0.22 ± 0.01 for group B and the average body weight of 67.37 ± 2.14 g, GSI = 0.31 ± 0.05% and sperm sac/testis = 0.52 ± 0.02 for group C (Table 2.1).

For proteomics based on one dimensional gel electrophoresis (SDS-PAGE) and nanoLC-MS/MS, wild male *P. monodon* broodstock (*N* = 6) were divided to two groups according to the SDS-PAGE protein patterns (*N* = 3 for each group with the average body weight of 123.55 ± 9.36 g, GSI = 0.66 ± 0.18% and sperm sac/testis = 0.51 ± 0.09 for group A and the average body weight of 120.67 ± 11.09g, GSI = 0.68 ± 0.09% and sperm sac/testis = 0.49 ± 0.06 for group B, respectively). In addition, domesticated broodstock: 14-month-old (*N* = 3, average body weight = 69.84 ± 2.76 g and GSI = 0.37 ± 0.03% and sperm sac/testis = 0.50 ± 0.02 for group C) and 18-month- old (*N* = 3, average body weight = 82.18 ± 2.88 g and GSI = 0.37 ± 0.01% and sperm sac/testis = 0.44 ± 0.01 for group D) were also included in the experiments (Table 2.1)**.**

2.2.2 Total protein extraction

Approximately 0.5 gram of the frozen testes of *P. monodon* were ground to fine powder in the presence of liquid N_2 and suspended in 10% TCA in acetone including 0.1% DTT and left at -20ºC for 1 hour. After centrifugation at 10000 g for 30 minutes at 4° C, the supernatant was discarded and the protein pellets were washed three times with the acetone solution before centrifuged at 10000 g for 30 minutes at 4° C. The pellet was air-dried and dissolved in the lysis buffer (30 mM Tris base, 2 M Thiourea, 7 M Urea, 4% CHAPS). The amount of extracted protein was measured by a Lowry-Peterson method (1977).

Table 2.1 Wild and domesticated broodstock of *P. monodon* used for proteomic analysis in this study

Sample	BD (g)	TT (g)	SP (g)	GSI (TT)	SP/TT
Specimens for proteomics based on two dimensional gel electrophoresis					
Wild broodstock (group A)					
BFNMTT4	160.37	1.51	0.31	0.94	0.21
BFNMTT6	151.18	2.17	0.45	1.44	0.21
BFNMTT10	105.09	0.9	0.34	0.86	0.38
	138.88±17.10			1.08 ±0.18%	0.26 ± 0.06
Domesticated broodstock (14-month-old, group B)					
BU14 M TT 41	66.05	0.20	0.04	0.30	0.20
BU14 M TT 20	60.22	0.21	0.05	0.35	0.24
BU14 M TT 19	58.84	0.27	0.06	0.46	0.22
	61.70 ± 2.1			0.37 ± 0.05%	0.22 ± 0.01
Domesticated broodstock (14-month-old, group C)					
BU14 M TT 39	70.10	0.17	0.09	0.24	0.53
BU14 M TT 13	68.87	0.20	0.11	0.29	0.55
BU14 M TT 28	63.15	0.25	0.12	0.40	0.48
	67.37 ± 2.14			0.31 ±0.05%	0.52 ± 0.02
Sample	**BD (g)**	**TT (g)**	**SP (g)**	**GSI (TT)**	**SP/TT**
Specimens for proteomics based on one dimensional gel electrophoresis					
Wild broodstock (group A)					
BFNMTT01	108.43	1.08	0.39	1.00	0.36
BFNMTT02	121.54	0.46	0.31	0.38	0.67
BFNMTT05	140.68	0.85	0.41	0.60	0.48
	123.55 ± 9.36			0.66 ±0.18%	0.51±0.09
Wild broodstock (group B)					
BFNMTT08	142.13	0.76	0.39	0.53	0.51
BFNMTT09	114.79	0.73	0.42	0.64	0.58
BFNMTT10	105.09	0.9	0.34	0.86	0.38
	120.67 ± 11.09			0.68 ± 0.09%	0.49 ± 0.06
Domesticated broodstock (14-month-old, group C)					
BU14 M TT 11	75.30	0.25	0.13	0.33	0.52
BU14 M TT 15	66.45	0.28	0.15	0.42	0.54
BU14 M TT 30	67.77	0.24	0.11	0.35	0.46
	69.84 ± 2.76			0.37 ± 0.03%	0.50 ± 0.02
Domesticated broodstock (18-month-old, group D)					
BU18 M TT 12	77.13	0.28	0.12	0.36	0.43
BU18 M TT 30	82.32	0.33	0.15	0.40	0.45
BU18 M TT 34	87.10	0.31	0.14	0.36	0.45
	82.18 ± 2.88			0.37 ±0.01%	0.44 ± 0.01

BD = body weight; TT = testicular weight; SP = weight of spermatophore; GSI = gonadosomatic index: (testicular weight/body weight) x 100, SP/TT = a ratio between weight of spermatophore and that of testes.

2.3 Determination of protein concentration by a Lowry-Peterson protein determination method

The protein pellet was resuspended in the lysis buffer and protein concentration was determined by a Lowry-Peterson protein determination method. The volume of protein samples (usually 20-50 µl) were adjusted with H_2O to the final volume of 1 ml. The 0.15% deoxycholate were added (0.1 ml), vortexed and kept at room temperature for 10 minutes. Subsequently, 72% TCA (100 µl) were added, vortexed and kept at room temperature for 10 minutes. The mixture was centrifuged at 13000 rpm for 25 minutes at 4° C, dissolved in the reagent A (50 µl) and kept at room temperature for 30 minutes. The reagent B (200 µl) was added and the reaction mixture was stood for 30 minutes at room temperature. The absorbance at 750 nm (OD_{750}) of each sample was measured by a spectrophotometer. The protein concentration of each sample was calculated using the standard curve, plotted between OD_{750} on the Y-axis and BSA concentration (µg/ml) on the X-axis.

2.4 Two-dimensional gel electrophoresis (2-DE)

2.4.1 Sample preparation

One hundred micrograms of total proteins were added to the rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS and 0.002% bromophenol blue) containing 4 mg DTT and 2% IPG in a total volume of 360 µl. The sample solution was vortexed and left in the dark for 30 minutes before centrifugation at 13,000 g for 15 minutes at 4 ºC.

2.4.2 Isoelectric focusing

The first dimension of 2-DE gel, isoelectric focusing (IEF), was performed using a 18 cm Immobiline Drystrip gel (GE Healthacre) linear pH gradient strips 3-10 and in an integrated system, the Ettan IPGphor III. The sample solution was applied in the strip holder. An IPG strip was then placed on the top of the sample and covered with a dry strip cover, after rehydrated for 12 hours in the IPGphor III. IEF was performed using the following the step voltage focusing protocol: pH 3-10; 500 V for

500 Vh, 1000V for 800 Vh, 8000V for 13,500 Vh, 8,000V for 12,200 Vh. All the above processes were carried out at 20°C.

2.4.3 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

After the first dimension, the IPG strip was equilibrated in the equilibration buffer (50 mM Tris-HCl pH 8.8, 6M urea, 30% glycerol, 2% SDS and bromophenol blue 200 ml) containing 1% DTT for 15 minutes. The IPG strip gel was removed to another equilibration buffer containing 2.5 % iodoacetamide and equilibrated for a further 15 minutes. The equilibrated IPG strip was then placed on the top of 12.5% polyacrylamide gel (40 % acrylamide in Tris-HCl pH 8.8, 10% SDS). The second dimension separation was electrophoresed initially at 2.5 W per gel for 30 minutes followed by 20 W/gel at 20°C for 3-4 hours.

2.4.4 Silver staining

At the end of each run, the gel protein was fixed in the fixing solution (50% methanol, 12% acetic acid and 50 µl of 37% formaldehyde to 100 ml fixing solution) for 2 hours. The gel was removed in the washing solution (35% ethanol) 3 times for 20 minutes each and sensitizing in 0.02% sodium thiosulfate for 2 minutes. After washing in water 3 times for 5 minutes each, the gel was stained with silver nitrate (2%) for 20 minutes and immersed in water for 30 seconds. The gel was shaken in the developing solution (60% $NaCO_3$ w/v, 0.04% $Na_2S_2O_3$ v/v, 37% formaldehyde CH_2O) until regarded protein spots were visualized and stopped quickly in the stopping solution (14.6% w/v sodium EDTA $C_{10}H_{12}N_2Na_4O_8$) for 20 minutes. The gel was scanned by a GS-710 Imaging Densitometer (BioRad). Gel image matching was carried out using Melanie gel analysis and the gel was kept in 0.1% acetic acid at 4ºC.

2.5 Mass spectrometry analysis

2.5.1 In-gel digestion

After protein spots were excised, the gel pieces were subjected to in-gel digestion using an in-house method developed by Proteomics Laboratory, Genome Institute, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand

(Jaresitthikunchai et al., 2009). Briefly, the spots were washed 3 times with 3% hydrogen peroxide and water, respectively. The gel plugs were dehydrated with 100% acetonitrile (ACN), reduced with 10mM DTT in 10mM ammonium bicarbonate at room temperature for 1 hour and alkylated at room temperature for 1 hour (dark) in 100 mM iodoacetamide (IAA) supplemented with 10 mM ammonium bicarbonate. After alkylation, the gel pieces were dehydrated twice with 100% ACN for 5 minutes. To perform in-gel digestion of proteins, 10 µl of trypsin solution (10 ng/µl trypsin in 50% ACN/10mM ammonium bicarbonate) was added to the gels followed by incubation at room temperature for 20 minutes, and then 20 µl of 30% ACN was added to keep the gels immersed throughout digestion. The gels were incubated at 37°C for a few hours or overnight. To extract peptide digestion products, 30 µl of 50% ACN in 0.1% formic acid (FA) was added into the gels, and then the gels were incubated at room temperature for 10 minutes in a shaker for three times. Peptides extracted were collected and pooled together in the new tube. The pool extracted peptides were dried by incubated at 40°C for 3-4 hours and kept at -80°C for further mass spectrometric analysis.

2.5.2 NanoLC-MS/MS

Nano-electrospay liquid chromatography ionization tandem mass spectrometry (nanoLC-MS/MS) was performed as followed. Selected protein spots were submitted to the HCTultra ETD II system™ (Bruker Daltonics). This system was controlled by the Chromeleon Chromatography Management system and comprised a two-pump Micromass/Loading Iontrap system with an autosampler. Injected samples were first trapped and desalted on an AccLaim PepMap C18 µ Precolumn Cartridge (5 µm, 300-µm inside diameter by 5 mm) for 3 minutes with 0.1% formic acid delivered by a loading pump at 20 µl/minutes, after which the peptides were eluted from the pre-column and separated on a nano column, AccLaim PepMap 100 C18 (15 cm x 3 µm) connected in-line to the mass spectrometer, at 300 nl/minutes using a 30 minutes fast gradient of 4 to 96% solvent B (80% acetronitrile in 0.1% formic acid).

2.5.3 Database searches

After data acquisition, MS/MS ion from nanoLC-MS/MS were identified using MASCOT (http://www. matrixscience.com) searched against data of the local

shrimp database. In addition, data from nanoLC-MS/MS were searched against data of the National Central for Biotechnology Information (NCBI, nr). For MS/MS ion search, the peptide charge was 1+, 2+ and 3+, MS/MS ion mass tolerance was ±1.2 Da, fragment mass tolerance ± 0.6 Da, and allowance for 1 miss cleavage. Variable modification was methionine oxidation and cysteine carbamidomethylation. Proteins with the highest score or higher significant scores were selected. The significant hit proteins were selected according to Mascot probability analysis and regarded as positive identification after additional conformation with molecular weight (MW)/ isoelectric point (p*I*) values

2.6 One dimensional polyacrylamide gel electrophoresis

2.6.1 Prefractionation of testicular proteins by SDS-PAGE

Proteins were size-fractionated on SDS-PAGE mini slab gel (BioRad.). The polyacrylamide gel was prepared according to the standard method described by Laemmli (1970). The SDS-PAGE gels (containing 5.0% stacking gel and 12.5% separating gel) were used for the fractionation of soluble proteins from testis. Ten micrograms of the protein samples were mixed with 5 µl of 5X sample buffer (0.125 M Tris-HCl; pH 6.8, 20% glycerol, 4% SDS, 0.2M DTT, 0.02% bromophenol blue), boiled at 95^{o}C for 10 minutes before loading onto the SDS-PAGE gel. To estimate size of polypeptides, low molecular weight protein standard marker (BioRad) was used. Electrophoresis was performed in SDS electrophoresis buffer (25 mM Tris-HCl pH 8.3, 192 mM glycine, 0.1% SDS) until the tracking dye reached the bottom of the gel. After the electrophoresis finished, gels were silver stained.

2.6.2 Silver staining

At the end of each run, the gel protein was fixed in the fixing solution (50% methanol, 12% acetic acid and 50 µl of 37% formaldehyde to 100 ml fixing solution) for 30 minutes. The gel was removed in the washing solution (35% ethanol) 2 times for 5 minutes each and sensitizing in 0.02% sodium thiosulfate for 2 minutes. After washing in water 2 times for 5 minutes each, the gel was stained with silver nitrate (2%) for 20 minutes and immersed in water for 30 seconds. The gel was shaken in the developing solution (60% $NaCO_3$ w/v, 0.04% $Na_2S_2O_3$ v/v, 37% formaldehyde CH_2O) until regarded protein pattern were visualized and gel was placed quickly in the

stopping solution (14.6% w/v sodium EDTA $C_{10}H_{12}N_2Na_4O_8$) for 20 minutes. The gel was scanned by a GS-710 Imaging Densitometer (Bio Rad) and the gel was kept in 0.1% acetic acid at 4ºC.

2.6.3 In-gel digestion

After protein bands were excised according to marker range (<20, 20-25, 25-37, 37-50, 50-75, 75-100, 100-250 and > 250 KDa), 3-4 pieces of approximately 1 square millimeter of gel pieces each were subjected to in-gel digestion described in section 2.5.1 with the exception that 20 µl of the trypsin solution (10 ng/µl trypsin in 50% ACN/10mM ammonium bicarbonate) was added to the gels.

2.6.4 HCTUltra LC-MS analysis

The protein digest was injected into Ultimate 3000 LC System (Dionex, USA) coupled to ESI-Ion Trap MS (HCT Ultra PTM Discovery System (Bruker, Germany) with electrospray at a flow rate of 300 nl/minutes to a nanocolumn (Acclaim PepMap 100 C18, 3 um, 100A, 75 um id x 150 mm). A solvent gradient (solvent A: 0.1% formic acid in water; solvent B: 80% 0.1% formic acid in 80% acetonitrile was run in 40 minutes.

2.6.5 Proteins quantitation and identification

Protein quantitation was carried out using DeCyder MS Differential Analysis software (DeCyderMS, GE Healthcare (Johansson et al., 2006; Thorsell et al., 2007)). Acquired LC-MS raw data were converted and the PepDetect module was used for automated peptide detection, charge state assignments, and quantitation based on the peptide ions signal intensities in MS mode. The analyzed MS/MS data from DeCyderMS were submitted to database search using the Mascot software (Matrix Science, London, UK, (Perkins et al., 1999)). The data was searched against the local shrimp database and searched against the NCBI database for protein identification. Database interrogation was; taxonomy(metazoa); enzyme (trypsin); variable modifications (carbamidomethyl, oxidation of methionine residues); mass values (monoisotopic); protein mass (unrestricted); peptide mass tolerance (1 Da); fragment mass tolerance (± 0.4 Da), peptide charge state (1+, 2+ and 3+) and max missed

cleavages (1). Proteins considered as identified proteins had at least two peptides with an individual mascot score corresponding to $P < 0.05$ and $P < 0.1$, respectively.

2.7 RNA extraction

Total RNA was extracted from ovaries and testes of each the shrimp using TRI REAGENT® or TriPure Isolation Reagent. A piece of tissues 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 TRI REAGENT® or TriPure Isolation Reagent (1 ml / 50–100 mg tissue) and homogenized. Additional 500 µl of TRI REAGENT or TriPure Isolation Reagent were added. The homogenate and left for 5 minutes, before 0.2 ml of chloroform was added. The homogenate was vortexed for 15 seconds and left at room temperature for 2 - 15 minutes and centrifuged at 12000g for 15 minutes at 4 °C. The mixture was separated into the lower red, phenol-chloroform phase, the interphase, and the colorless upper aqueous phase. The aqueous phase (inclusively containing RNA) was carefully transferred to a new 1.5 ml microcentrifuge tube. RNA was precipitated by an addition of 500 µl of isopropanol and mixed thoroughly. The mixture were left at room temperature for 10-15 minutes and centrifuged at 12000g for 10 minutes at 4 - 25 °C. The supernatant was removed. The RNA pellet was washed with 1 ml of 75% ethanol and centrifuged at 7500g for 5 minutes at 4 °C. The ethanol was removed. The RNA pellet was air-dried for 5 – 10 minutes. RNA was dissolved in DEPC-treated H_2O for immediately used. Alternatively, the RNA pellet was kept under absolute ethanol in a -80 ° C freezer for long storage.

Total RNA was also extracted from other tissues including antennal gland, eyestalks, gills, heart, hemocytes, hepatopancreas, lymphoid organs, intestine, stomach, pleopod and thoracic ganglion of *P. monodon* using the same extraction procedure. The quality of extracted total RNA was examined by electrophoresed through 1.2% agarose gels.

2.8 DNase I treatment of the extracted RNA

Fifteen micrograms of total RNA were treated with DNase I (0.5 U/1 µg of RNA, Promega) at 37ºC for 30 minutes. After the incubation, the sample was gently

mixed with a sample volume of phenol:chloroform:isoamylalcohol (25:24:1) for 10 minutes. The mixture was centrifuged at 12,000 g for 10 minutes at 4ºC, and the upper aqueous phase was collected. The extraction process was then repeated once with chloroform:isoamylalcohol (24:1) and one with chloroform. The final aqueous phase was mixed with one-tenth final sample volume of 3 M sodium acetate (pH 5.2). After that, RNA was precipitated by adding two point five volume of -20°C-cold absolute ethanol. The mixture was incubated at -80°C for 30 minutes, and the precipitated RNA was recovered by centrifugation at 12,000 g for 10 minutes at room temperature. The RNA pellet was then washed twice with 1 ml of -20°C cold 75% ethanol. Alternatively, the RNA pellet was kept in absolute ethanol at -80°C until required.

2.9 Estimation of total RNA concentration

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

[Nucleic acid] = OD_{260} x dilution factors x nucleotide factor; nucleotide factor = 40 or 33 for 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 RNA solution. The ratio between OD_{260}/OD_{280} provides an estimate on the purity of extracted RNA. For the extracted RNA, a pure preparation of RNA 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 RNA (Sambrook and Russell, 2001).

2.10 Examination of expression patterns of gene homologues in *P. monodon* by RT-PCR and tissue distribution analysis

2.10.1 Primer design

Eight pairs of primers were designed from EST sequences of gene homologues from testis and hemocyte cDNA libraries of *P. monodon* Generally, the PCR primers is 20-24 bp in length with melting temperatures of 60-70^{o}C and the GC content of 40-50% (Table 2.2).

2.10.2 First strand cDNA synthesis

One and half micrograms of total RNA from various tissues of *P. monodon* were reverse transcribed to the first strand cDNA using an ImProm-II^{TM} Reverse Transcription System Kit (Promega). Total RNA was combined with 0.5 µg of oligo dT_{12-18} and appropriate amount of DEPC-treated H_2O in a final volume of 5 µl. The reaction was incubated at 70 ºC for 5 minutes and immediately placed on ice for 5 minutes. The 5x reaction buffer, $MgCl_2$, dNTP mix, RNasin were added to final concentration of 1x, 2.25 mM, 0.5 mM and 20 units, respectively. Finally, 1 µl of ImProm-II^{TM} Reverse transcriptase was added and gently mixed by pipetting. The reaction mixture was incubated at 25 ºC for 15 minutes and 42 ºC for 90 minutes. The reaction was terminated by incubated at 70 ºC for 15 minutes to terminate reverse transcriptase activity. Concentration and rough quality of newly synthesized first strand cDNA was spectrophotometrically examined (OD_{260}/OD_{280}) and electrophoretically analyzed by 1.2% agarose gel.

2.10.3 RT-PCR analysis

Two hundred nanograms of the first strand cDNA of testes of male broodstock-sized *P. monodon* were used as the template in a 25 µl RT-PCR reaction composing of 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.1% Triton X-100, 100 mM of each dNTP, 2 mM $MgCl_2$, 0.2 µM of each primer and 1 unit of Dynazyme ^{TM} DNA polymerase (Finnzymes). RT-PCR was carried out with the temperature profile of predenaturation at 94 ºC for 3 minutes followed by 30 cycles of denaturation at 94 ºC for 30 seconds, annealing at 55 ºC for 45 seconds and extension at 72 ºC for 45 seconds. The final extension was carried out at the same temperature for 7 minutes.

Fives microliters of the amplification products are electrophoresed though 1.2-2.0% agarose gel dependent on sizes of the amplification products. The electrophoresed band was visualized under a UV transilluminator after ethidium bromide staining (Sambrook and Russell, 2001).

2.10.4 Agarose gel electrophoresis

An appropriate amount of agarose was weighed out and mixed with the desired volume of 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid and 2 mM EDTA, pH 8.3). The gel slurry was boiled in a microwave oven to complete solubilization and allowed to lower than 60 ºC before poured into the gel mold. A comb was inserted. The agarose gel was left to solidify. When needed, enough amount of 1x TBE buffer covering the gel for approximately 0.5 cm. The comb was removed. The PCR product was mixed with the one-fourth volume of the 10x loading dye (0.25% bromophenol blue and 25% ficoll in water) and loaded into the well. A 100 bp DNA ladder was used as the standard DNA marker. Electrophoresis was carried out at 5-6 volt/cm until bromophenol blue moved to approximately one-haft of gel. The electrophoresed gel was stained with an ethidium bromide solution (25 µg/ml) for 5 minutes and destained in running tap water to remove unbound ethidium bromide from the gel. DNA fragments were visualized under a UV transilluminator and photographed through a Gel Doc using a Quality One software (BioRad).

2.11 Tissue distribution analysis

Total RNA was extracted from eyestalk, gills, heart, hemocytes, hepatopancrease, lymphoid organ, intestine, pleopods, stomach, testes, thoracic ganglion and epicuticle of a male and ovaries of a female *P. monodon* broodstock. The first strand cDNA was synthesized as described previously For the target genes, 200 ng of the first strand cDNA from various tissues was used as the template in a 25 µl reaction volume containing 10 mM Tris-HCl, pH 8.8, 50 mM KCl and 0.1% Triton X-100, 2 mM $MgCl_2$, 100 µM each of dATP, dGTP, dTTP and dCTP, 0.2 µM of each primer and 1 unit of Dynazyme ™ DNA polymerase (FINNZYMES). *Elongation factor-1α* (F: 5′-ATGGTTGTCAACTTTGCCCC-3′ and R: 5′-TTGACCTCCTTGATCACACC-3′) were also amplified from the same template and considered as the positive control.

The reactions were predenaturation at 94 ºC for 3 minutes followed by 30 cycles composing of a 94 ºC denaturation step for 45 s, a 55 ºC annealing step for 45 s and 72 ºC extension step for 45 s. The final extension was carried out at 72 ºC for 7 minutes. Fives microliters of the amplification product was electrophoretrically analyzed though a 1.5% agarose gel.

Table 2.2 Gene homologue, primer sequences and expected sizes of the PCR product designed from EST of *P. monodon*. and 2 dimensional gel electrophoresis.

Gene/Primer	Sequence	Tm (ºC	Size Bp
1. *Ubiquitin carboxyl-terminal hydrolase 14*	F: 5'ACAGTTCTGATGATGGGGAGCA3'	62	227
	R: 5'CCAGGAGGCTTGGGCTTGAA3'	60	
2. *Ubiquitin carboxyl-terminal hydrolase 5*	F: 5'CAAGTTGGCTGCCCCTGAAG3'	60	528
	R: 5' GTTGCCTGCTCTCGTGTGAATC 3'	68	
3. PCTAIRE protein kinase 2 (Cdk 17)	F: 5'CGAGACCTCAAGCCTCAGAACC3'	70	250
	R: 5'CTCTTCCCAGGTGCCACAGTAG3'	70	
4. *Serine/threonine-protein kinase 23*	F: 5'ATGGTGTTTGAAGTGCTGGGTC3'	66	229
(*Muscle-specific serine kinase 1*) (*MSSK-1*)	R: 5'CTTATGAGGCAACCCAGTGGC3'	66	
5. *ubiquitin conjugating enzyme 2*	F: 5'TCTGCCTCGCCTGCTGGT3'	60	232
	R: 5'ATGTCAAAGGCACTCAGCACCA3'	66	
6. *Dynein light intermediate chain*	F: 5'GCAAGTCTGTTCTCGTCCTGG 3'	66	324
	R: 5'TGTCTATGTGGTCTTGGAGAGTGG3'	70	
7.*proteasome alpha subunit, putative*	F: 5'AAAGATGGTGTTGTGTTTGCTGTAG3'	68	250
	R: 5'CCTACCTTCATGCCTATACCCTCT3'	66	
8. *proteasome delta*	F: 5'GCTAGGAACTTACGTCTCAAATC3'	70	146
	R: 5'GCTTCACCTGTAGAATCTCCAT3'	64	

2.12 Isolation and characterization of the full length cDNA of functionally important gene homologues of *P. monodon* using Rapid Amplification of cDNA Ends-Polymerase Chain Reaction (RACE-PCR)

2.12.1 Preparation of the 5' and 3' RACE template

Full length cDNAs of interesting gene homologues were further characterized using a SMART RACE cDNA Amplification Kit (Clontech). Total RNA was extracted from testis of *P. monodon* using TriPure (Roche). The quality of extracted of total RNA was determined by agarose gel electrophoresis. Messenger (m) RNA

was purified using a QuickPrep micro mRNA Purification Kit (GE Healthcare) according to the protocol recommended from the manufacturer. RACE cDNA template was prepared by combining 1 µg of testis mRNA with 1 µl of 5'-CDS primer and 1 µl of 10 µM SMART II oligonucleotide for 5' RACE-PCR or 1 µg of testis mRNA with 1 µl of 3' CDS primer A for 3' RACE-PCR (Table 2.3). The component were mixed and centrifuged briefly. The reaction was incubated at 70ºC for 2 minutes and snap-cooled on ice for 2 minutes. The reaction tube was centrifuged briefly. After that, 2 µl of 5x First-Strand buffer, 1 µl of 20 mM DTT, 1 µl of dNTP Mix (10 mM each) and 1 µl of PowerScript Reverse Transcriptase were added. The reaction were mixed by gently pipetting and centrifuged briefly to collect the contents at the bottom of the tube. The reaction tube was incubated at 42 ºC for 1.5 h in a thermocycler. The first strand reaction products were diluted with 125 µl of TE buffer and heated at 72 ºC for 7 minutes. The first strand cDNA template was kept at -20 ºC until needed.

2.12.2 Primer designed for RACE-PCR

Gene-specific primers (GSPs) were designed from testis and cDNA libraries. The antisense/sense primers were design for 5′ and/or 3′ RACE-PCR, respective (Table 2.4). For sequencing of genes that showed the full length from the 5′ direction, the product from colony PCR was considered. If the insert of a particular gene was larger than that of its homologues, the 3′ direction was further sequenced. Internal primers were designed for primer walking of the inserted cDNA (Table 2.4).

2.12.3 RACE-PCR

The master mix sufficient for 5′ and/or 3′ RACE-PCR and the control reactions was prepared (Tables 2.5 and 2.6). For each 25 µl amplification reaction, 16.0-17.0 µl sterile deionized H_2O, 2.5 µl of 10x Advantage® 2 PCR buffer, 0.5 µl of 10 uM dNTP mix and 0.5 µl of 50x Advantage® 2 polymerase mix were combined. The reaction was carried out for as described in Tables 2.5 and 2.6.

The primary 5′ and 3′ RACE-PCR product were electrophoretically analyzed through 1.2-1.5 % agarose gels. If the discrete expected bands were not obtained from the primary amplification, nested PCR was performed using the recipes illustrated in Tables and The primary PCR product was 50-fold diluted. The secondary PCR was

performed using 1 - 5 µl of the diluted first PCR product (50 fold) as a template using the conditions described in Table 2.7.

Table 2.3 Primer sequence for the first strand cDNA synthesis and RACE-PCR

Primers	Sequence
SMART II A Oligonucleotide	5′-AAGCAG TGG TATCAACGCAGAGTACGC GGG-3′
3′ RACE CDS Primer A	5′-AAGCAGTGGTATCAACGCAGAGTAC$(T)_{30}$ N_{-1} N-3′ (N=A, C, G orT; N_{-1}= A,G or C)
5′ RACE CDS Primer	5′-$(T)_{25}$ N_{-1} N-3′ (N=A, C, G orT; N_{-1}= A,G or C)
10X Universal PrimerA Mix (UPM)	Long : 5′-CTAATACGACTCACTATAGGGCAA GCAGTGGTATCAACGCAG AGT-3′
	Short : 5′-CTAATACGACTCACTATAGGG C-3′
Nested Universal Primer A (NUP)	5'-AAG CAG TGG TAT CAA CGC AGA GT -3′

Table 2.4 Gene-specific primers (GSPs) and nested GSP used for isolation of the full length cDNA of functionally important genes in *P. monodon*

Gene specific primer	Sequence	Tm (°C)
ubiquitin specific peptidase 14		
3′ RACE	F: 5'ACAGTTCTGATGATGGGGAGCA3'	62
Ubiquitin carboxyl-terminal hydrolase 5		
5'RACE	R: 5'ACCATGTCGTCTTCGTCATAACTG3'	68
3′ RACE	F: 5'CAAGTTGGCTGCCCCTGAAG3'	60
PCTAIRE protein kinase 2 (*Cdk17*)		
5'RACE	R: 5'AGAACTCGCCGCTGGTGGCAATAGG3'	66
3′ RACE	F: 5'TGCCAAGTCAGTGCCAACCAAGA3'	70
Dynein light intermediate chain		
5'RACE	R:TCACCCAGGACGAGAACAGACTT3'	70
3'RACE	F: 5'GCAAGTCTGTTCTCGTCCTGG 3'	66
3'RACE internal F	F: 5'CATTCAGAACCCCAGCACTTG3'	64
3'RACE internal R	R:5'GACGACCTTGTGTAGCATTGG3'	64
Proteasome alpha subunit, putative		
3'RACE	F: 5'AAAGATGGTGTTGTGTTTGCTGTAG3'	70
26S proteasome regulatory subunit S3		
5'RACE	R: 5'ACTTACTATGGGCGACCAGAGAA3'	68
3'RACE	F: 5'CGCCTGGTTGAACGCAGCATTG3'	70

Table 2.5 Compositions for amplification of the 5′ end of gene homologues using 5′ RACE-PCR

Component	5′ RACE-PCR	UPM only (Control)	GSP1 only (Control)
5’ RACE–Ready cDNA template	2.0 µl	2.0 µl	2.0 µl
UPM (10x)	2.5 µl	2.5. µl	-
GSP1 (10 uM)	0.5-1.0 µl	-	0.5-1.0 µl
GSP2 (10 uM)	-	-	-
H_2O	-	0.5-1.0 µl	2.5 µl
Master Mix	20 -19.5 µl	20 -19.5 µl	20 -19.5 µl
Final volume	25 µl	25 µl	25 µl

Table 2.6 Compositions for amplification of the 3′ end of gene homologues using 3′ RACE-PCR

Component	3′ RACE-PCR	UPM only (Control)	GSP1 only (Control)
5’ RACE–Ready cDNA template	2.0 µl	2.0 µl	2.0 µl
UPM (10x)	2.5 µl	2.5 µl	-
GSP1 (10 uM)	0.5-1.0 µl	-	0.5-1.0 µl
GSP2 (10 uM)	-	-	-
H_2O	-	0.5-1.0 µl	2.5 µl
Master Mix	20 -19.5 µl	20 -19.5 µl	20 -19.5 µl
Final volume	25 µl	25 µl	25 µl

Table 2.7 The amplification conditions for RACE-PCR of various gene homologues of *P. Monodon*

Gene homologue	Amplification condition
Ubiquitin carboxyl-terminal hydrolase 14	
3′ RACE	25 cycles of 94 °C for 30 s, 65°C for 45 s, 72 °C for 2 min and the final extension at 72 °C for 7 min
Ubiquitin carboxyl terminal hydrolase 5	
5′ RACE-PCR	25 cycles of 94 °C for 30 s, 68 °C for 45 s, 72 °C for 90s and the final extension at 72 °C for 7 min
semi nested 5′ RACE-PCR	25 cycles of 94 °C for 30 s, 68 °C for 45 s, 72 °C for 90s and the final extension at 72 °C for 7 min
3′ RACE-PCR	25 cycles of 94 °C for 30 s, 65 °C for 45 s, 72 °C for 2 min and the final extension at 72 °C for 7 min
PCTAIRE protein kinase 2	
5′ RACE-PCR	5 cycles of 94 °C for 30 s, 72 °C for 90s, 5 cycle of 94 °C for 30 s, 70 °C for 30 s, 72°C for 90s, 20 cycle of 94°C for 30 s, 68°C for 30 s, 72°C for 90s and the final extension at 72 °C for 7 min
semi nested 5′ RACE-PCR	25 cycles of 94 °C for 30 s, 65 °C for 45 s, 72 °C for 90s and the final extension at72 °C for 7 min
3′ RACE-PCR	20 cycles of 94 °C for 30 s, 68 °C for 45 s, 72 °C for 2 min and the final extension at 72 °C for 7 min
Dynein light intermediate chain	
5'RACE-PCR	5 cycles of 94 °C for 30 s, 72 °C for 1 min, 5 cycle of 94 °C for 30 s, 70 °C for 30 s, 72°C for 1 min, 20 cycle of 94°C for 30 s , 68°C for 30 s, 72°C for 1 min and the final extension at 72 °C for 7 min
3′ RACE-PCR	25 cycles of 94 °C for 30 s, 65 °C for 45 s, 72 °C for 90s and the final extension at 72 °C for 7 min
Proteasome alpha subunit, putative	
3′ RACE-PCR	5 cycles of 94 °C for 30 s, 72 °C for 90s, 5 cycle of 94 °C for 30 s, 70 °C for 30 s, 72°C for 90s, 20 cycle of 94°C for 30 s , 68°C for 30 s, 72°C for 90s and the final extension at 72 °C for 7 min
26S proteasome regulatory subunit S3	
5′ RACE-PCR	5 cycles of 94 °C for 30 s, 72 °C for 90s, 5cycle of 94 °C for 30 s, 70 °C for 30 s, 72°C for 90s, 20 cycle of 94°C for 30 s , 68°C for 30 s, 72°C for 90s and the final extension at 72 °C for 7 min
3′ RACE-PCR	5 cycles of 94 °C for 30 s, 72 °C for 90s, 5 cycle of 94 °C for 30 s, 70 °C for 30 s, 72°C for 90s, 20 cycle of 94°C for 30 s, 68°C for 30 s, 72°C for 90s and the final extension at 72 °C for 7 min

2.12.4 Elution DNA fragments from agarose gels

After electrophoresis, the desired DNA fragment was excised from the agarose gel using a sterile scalpel and placed in a pre-weighed microcentrifuge tube. DNA was eluted out from the gel using a HiYield™ Gel Elution Kit (RBC). Five hundred microlitres of the DF buffer was added to the sample and mixed by vortexing. The mixture was incubated at 55 °C for 10 - 15 minutes until the gel slice was completely dissolved. During the incubation period, the tube was inverted every 2-3 minutes. A DF column was placed in a collection tube and 800 µl of the sample mixture was applied into the DF column and centrifuged at 6,000 *g* (8,000 rpm) for 30s. The flow-through was discarded. The DF column was placed back in the collection tube. The column was washed by the addition of 500 µl of the ethanol-added Wash Buffer and centrifuged at 6,000 *g* for 30s. After discarding the flow-through, the DF column was centrifuged for 2 minutes at the full speed (14,000 rpm) to dry the column matrix. The dried column was placed in a new microcentrifuge tube and 15 µl of the Elution Buffer or water was added to the center of the column matrix. The column was left at room temperature for 2 minutes before centrifuged for 2 minutes at the full speed to recover the gel-eluted DNA.

2.12.5 Ligation of PCR product to pGEM®-T Easy vector

Ligation of PCR product to pGEM®-T Easy vector the ligation reaction was set in the total volume of 5 µl containing approximately 50 ng of the gel-eluted PCR product, 25 ng of pGEM®-T Easy vector, 2.5 ul of 2X rapid ligation buffer (60 mM Tris-HCL Ph 7.8, 20 mM $MgCl_2$, 2mM ATP and 10% PEG 8000) and 3 Weiss units of T4 DNA ligase. The ligation mixture was gently mixed by pipetting and incubating at 4°C overnight.

2.12.6 Transformation of the ligation product to *E.coli* host cells

2.12.6.1 Preparation of competent cells

A single colony of *E.coli* JM109 was inoculated in 5 ml of LB broth (1% Bacto tryptone, 0.5% Bacto yeast extract and 0.5% NaCl) with vigorous shaking at 37°C overnight. The starting culture was inoculated into 50 ml of LB broth and continued culture at 37°C with vigorous shaking to the OD_{600} of 0.5 to 0.8. The cells

were briefly chilled on ice for 10 minutes before centrifuged at 2,700 g for 10 minutes at 4°C. The pellets were resuspended in 30 ml of ice-cold $MgCl_2$-$CaCl_2$ solution (80mM $MgCl_2$ and 20 mM $CaCl_2$) and centrifuged as above. The supernatant was discarded and the pellet was resuspended in 2 ml of ice-cold 0.1 mM $CaCl_2$ and divided into 100 ul aliquots. These competent cells could be used immediately or store at 80°C for subsequently used.

2.12.6.2 Transformation

The competent cells were thawed on ice for 5 minutes. Two to four microlitres of the ligation mixture were added and gently mixed by pipetting. The mixture was incubated on ice for 30 minutes. During the incubation period, the ice box was gently moved forward and backward a few times every 5 minutes. The reaction tube was heat-shocked in 42ºC water bath for exactly 45 seconds without shaking. The reaction tube was then immediately snapped on ice for 2-3 minutes. One microlitre of SOC medium (2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM $NaCl_2$, 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 shaking at 37ºC for 1 to 1.5 hours. At the end on the incubation period, the cultured cell suspension was centrifuged at 6,000 rpm for 1 minute at room temperature. The pellet was gently resuspended in 100 µl of SOC and spread on a LB agar plate containing 50 µg/ml of ampicillin,25 µg/ml of IPTG and 20 µg/ml of X-gal. 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 insert DNA are blue (Sambrook and Russell, 2001).

2.12.6.3 Colony PCR

Colony PCR was performed in a 25 µl reaction volume containing 75 mM Tris-HCl (pH 8.8 at 25°C), 20 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2 mM $MgCl_2$, 100 µM each of dATP, dCTP, dGTP and dTTP, 0.1 µM of pUC1 (5' TCC GGC TCG TAT GTT GTG TGG A 3') and pUC2 (5' GTG CTG CAA GGC GAT TAA GTT GG 3') primers and 0.5 unit of *Taq* DNA Polymerase (Fermentus). A recombinant colony was picked up by the micropipette tip and mixed well in the amplification reaction. The PCR profiles was predenaturing at 94°C for 3 minutes, followed by 35 cycles of

94°C for 30 seconds, 50°C for 1 minute and 72 °C for 1-3 minutes. The final extension was carried out at 72°C for 7 minutes. The colony PCR products were electrophoretically analyzed through a 1.2 %-1.5% agarose gel and visualized after ethidium bromide staining.

2.12.6.4 Isolation and digestion of recombinant plasmid DNA

Plasmid DNA was isolated using a HiYield^{TM} Plasmid Mini Kit (RBC). A recombinant clone was inoculated into 3 ml of LB broth (1% tryptone, 0.5% yeast extract, 1.0 % NaCl) containing 50 μg/ml of ampicillin and incubated at 37°C with constant shaking at 250 rpm overnight. The culture was transferred into 1.5 ml microcentrifuge tube and centrifuged at 14,000 rpm for 1 minute. The supernatant was discarded. The bacterial cell pellet was collected and resuspended with 200 μl of the PD1 buffer containing RNaseA and thoroughly mixed by vortexed. The resuspended cells were lysed by the addition of 200 μl of the PD2 buffer and mixed gently by inverting the tube 10 times. The mixture was stood for 2 minutes at room temperature. After that, 300 μl of the buffer PD3 was added to neutralize the alkaline lysis step and mixed immediately by inverting the tube for 10 times. To separate the cell debris, the mixture was centrifuged at 14,000 rpm for 15 minutes. The supernatant was transferred into a new microcentrifuge tube and to the PD column and centrifuged at 6,000*g* (8,000 rpm) for 1 minute. The flow-through was discarded. The PD 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 minute. After discarding the flow-through, 600 μl of the ethanol-added Wash buffer was added and centrifuged as above. The flow-through was discarded. The spin tube was centrifuge for an additional 2 minute at full speed (14,000 rpm) to remove the residual Wash buffer. The dried PD column was placed in a new 1.5 ml microcentrifuge tube and 30-50 μl of the Elution buffer or water was added at the center of the column to elute the extracted plasmid DNA. The column was left at room temperature for 2 minutes and centrifuge at 14,000 rpm for 2 minutes. The concentration of extracted plasmid DNA was spectrophotometrically measured.

The insert size of each recombinant plasmid was also examined by digestion of the plasmid with *Eco R*I. The digest was carried out in a 12 μl containing 1x

restriction buffer (90 mM Tris-HCl; pH 7.5, 10 mM NaCl and 50 mM $MgCl_2$), 3 units *Eco R*I (Promega) and 1 µl of recombinant plasmid and incubated at 37°C for 4 hours or overnight before analyzed by agarose gel electrophoresis.

2.12.6.5 DNA sequencing

The recombinant plasmid was unidirectional sequenced using the M13 reverse or M13 forward primers on an automated DNA sequencer. Nucleotide sequences were blasted against data in the GenBank (http://www.ncbi.nlm.nih.gov/blast) using Blast*N* (a nucleotide-level annotation against the nucleotide collection, nr/nt, database) and Blast*X* (a protein-level annotation against the non-redundant protein sequences, nr, database).

2.13 Examination of expression levels of interesting genes in testis of *P. monodon* by quantitative real-time PCR

Expression levels of several transcripts including *ubiquitin carboxyl-terminal hydrolase 14*, *proteasome alpha subunit, putative, 26S proteasome regulatory subunit S3*, *serine/threonine-protein kinase 23* (*muscle-specific serine kinase 1*, *MSSK-1*) and *proteasome delta* were examined using quantitative real-time PCR analysis.

2.13.1 Experimental animals

Male juveniles of *P. monodon* (*N* = 3, average body weight 37.82 g, 6-month-old), domesticate broodstock: 10-month-old (*N* = 4, average body weight 51.24 ± 3.27 g and GSI = 0.7 ± 0.08), 14- month-old (*N* = 3, average body weight 62.40 ± 3.87 g and GSI = 0.38 ± 0.01), and 18-month-old (*N* = 5, average body weight 74.10 ± 4.28 g and GSI = 0.52 ± 0.06) and wild broodstock (*N* = 5, average body weight 133.62 ± 10.25 g and GSI = 0.7 ± 0.08) were used for real-time PCR analysis.

2.13.2 Primers and construction of the standard curves

For construction of the standard curve of each gene, the PCR product of the target gene and *EF-1α* was amplified using gene-specific primers described in Table 2.8, and electrophoretically analyzeed through agarose gels. The gel-eluted product was cloned into pGEM-Teasy vector and transformed into *E. coli* JM109. Plasmid DNA were extracted and used as the template for construction of the standard curve.

Templates of each gene homologues were ten fold diluted covering 10^2 – 10^8 copy numbers. For *EF-1α*, 10^3 – 10^8 copy numbers were used. Real-time RT-PCR was carried out (see below) and each standard point was run in duplicate.

Table 2.8 Gene homologue, primer sequences and expected sizes of the PCR product designed from EST of *P. monodon* for quantitative real-time PCR

Gene/Primer	Sequence	Tm (ºC)	Size bp
1. *Ubiquitin carboxyl-terminal hydrolase 1*4	F: 5'ACAGTTCTGATGATGGGGAGCA3'	62	227
	R: 5'CCAGGAGGCTTGGGCTTGAA3'	60	
2. *Serine/threonine-protein kinase 23 (Muscle-specific serine kinase 1) (MSSK-1)*	F: 5'ATGGTGTTTGAAGTGCTGGGTC3'	66	229
	R: 5'CTTATGAGGCAACCCAGTGGC3'	66	
3.*proteasome alpha subunit, putative*	F: 5'AAAGATGGTGTTGTGTTTGCTGTAG3'	68	250
	R: 5'CCTACCTTCATGCCTATACCCTCT3'	66	
4. *26S proteasome regulatory subunit S3*	F: 5'CGCCTGGTTGAACGCAGCATTG3'	70	140
	R: 5'ACTTACTATGGGCGACCAGAGAA3'	68	
5. *proteasome delta*	F: 5'GCTAGGAACTTACGTCTCAAATC3'	70	146
	R: 5'GCTTCACCTGTAGAATCTCCAT3'	64	

2.13.3 Quantitative real-time PCR

The first strand cDNA of each shrimp was reverse-transcribed. The target transcript *(ubiquitin carboxyl-terminal hydrolase 14*, *proteasome alpha subunit,, 26S proteasome regulatory subunit S3*, *proteasome delta* and *serine/threonine-protein kinase 23*) and internal control (*EF-1α*) of each shrimp were amplified in reaction volume 10 µl containing 5 µl of 2x SYBR Green Master Mix (Roch). The specific primer pairs were used at a final concentration of 0.3 µM for *ubiquitin carboxyl-terminal hydrolase 14* and *proteasome delta*, 0.25 µM for *proteasome alpha subunit, 26S proteasome regulatory subunit S3* and *serine/threonine-protein kinase 23*, respectively. The thermal profile for quantitative real-time RT-PCR was 95ºC for 10 minutes followed by 40 cycles of denaturation at 95 ºC for 15 s, 20 s, annealing at 53 ºC for 30 s and extension at 72 ºC for 30 s. Continually, cycles for the melting curve analysis was carried out at 95 ºC for 15 s, 65 ºC for 1 minute and at 98ºC for continuity and cooling 40 ºC for 30 s. Real-time RT-PCR assay was carried out in 96

well plate and each sample was run in duplicate Relative expression levels of different group of samples were statistically test by one way ANOVA followed by Duncan's new multiple rang test ($P < 0.05$).

CHAPTER III

RESULTS

3.1 Protein profiles of *P. monodon* testes examined by two dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2-DE) was carried out to examine protein profiles in testes of both wild and domesticated *P. monodon* broodstock. Initially, residual proteins after extraction of total RNA were isolated from *P. monodon* originating from Angsila (Chonburi, Gulf of Thailand) and subjected to two-dimensional gel electrophoresis. The conditions were further optimized. Subsequently, total proteins extracted from testis of wild and domesticated shrimp exhibiting different gonadosomatic index (GSI) and a ratio between the weight of sperm sac and that of testis were eventually used to identify and characterize testicular protein profiles of this economically important species.

3.1.1 Protein profiles during testicular development using total testes proteins

Total proteins extracted from different groups of *P. monodon* exhibiting different gonadosomatic index (GSI) and a ratio between the weight of testis to that of sperm sac were electrophoretically analyzed using the IEF gradient of pH 3-10 followed by 12.5% SDS-PAGE.

Two-dimensional gel electrophoresis of total testicular protein from wild *P. monodon* broodstock (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3; group A) and domesticated broodstock (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* =3; group B and GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.01, *N* =3; group C, respectively) at the broad pH gradient revealed that a large number of electrophoresed protein spot were found and almost all of the expressed testicular proteins were acidic proteins. Much lower numbers of basic proteins were observed in testis of *P. monodon* (Figures 3.1-3.9).

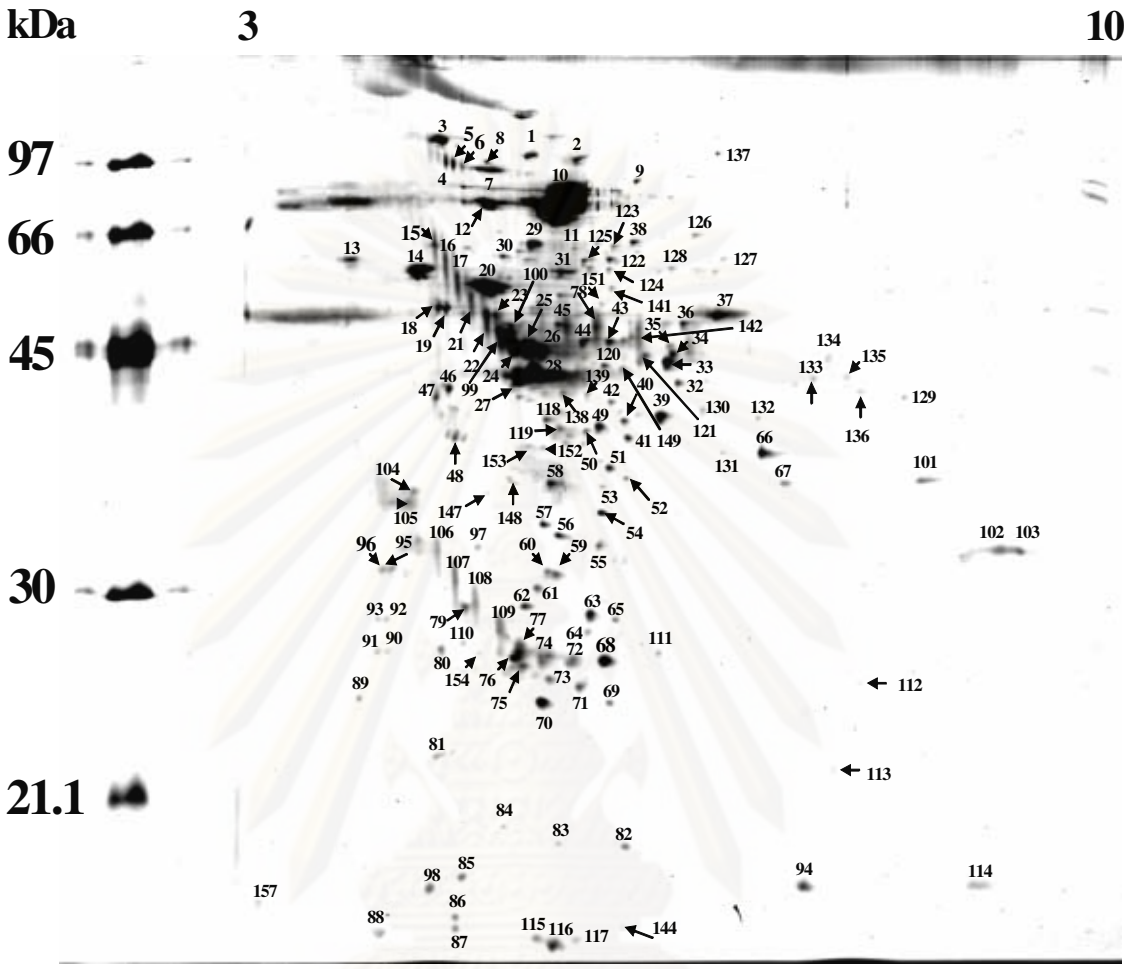


Figure 3.1 Protein profiles of testis of wild *P. monodon* broodstock (GSI = 0.94 and sperm sac/testis = 0.21; average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

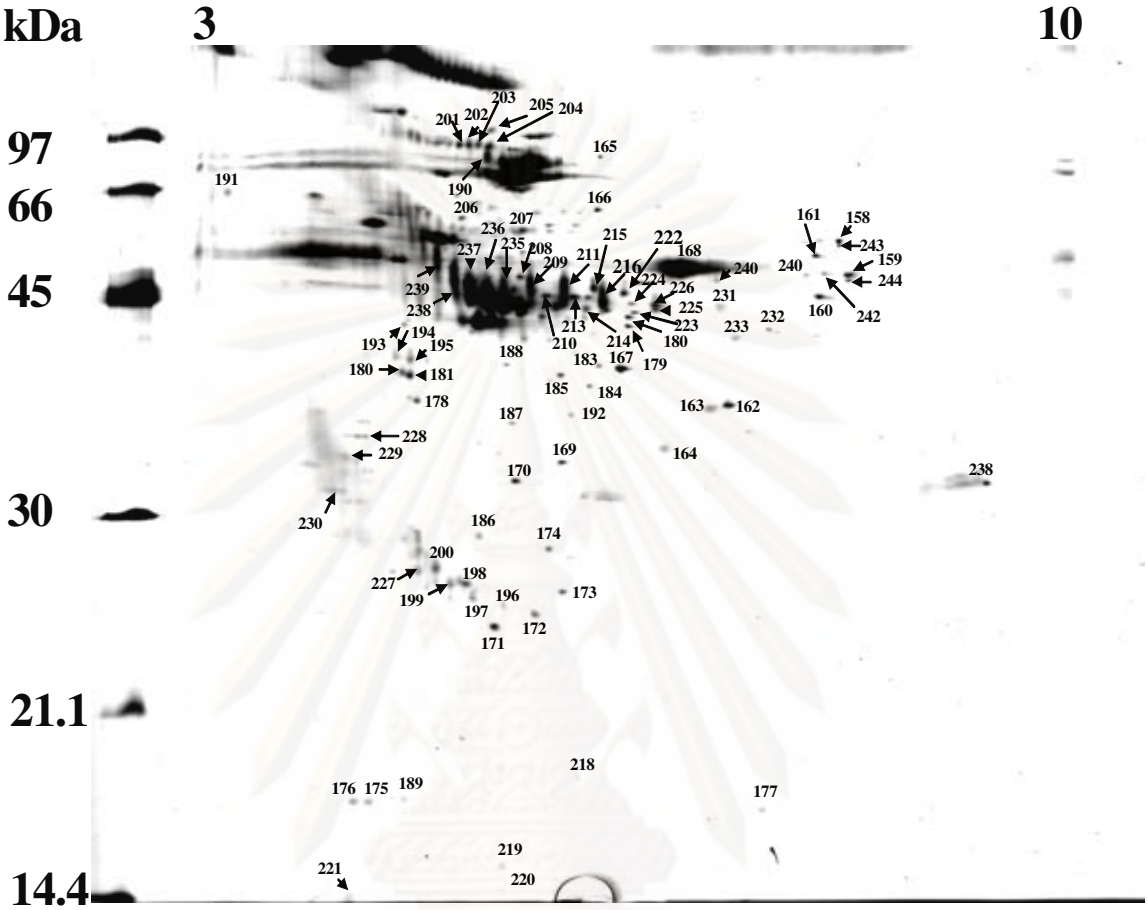


Figure 3.2 Protein profiles of of wild *P. monodon* broodstock (GSI = 1.44 and sperm sac/testis = 0.21; average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

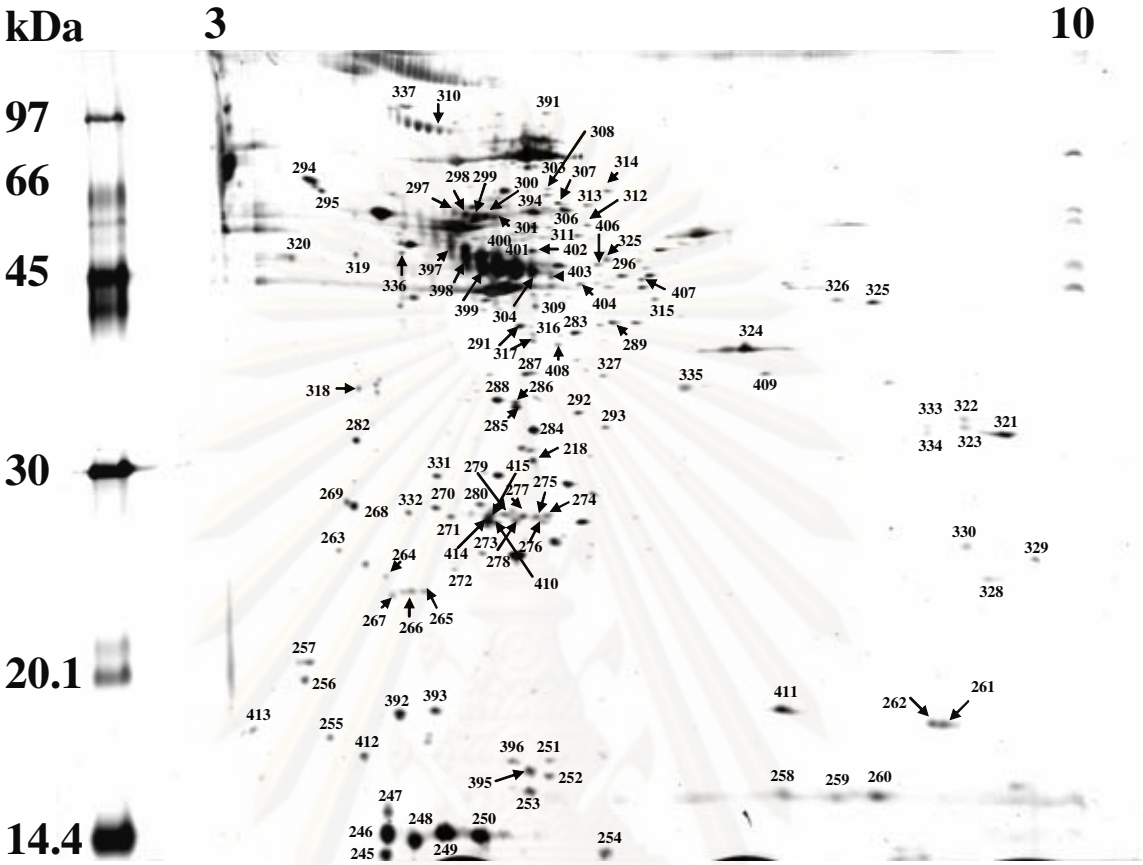


Figure 3.3 Protein profiles of of wild *P. monodon* broodstock (GSI = 0.86 and sperm sac/testis = 0.38; average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-710 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

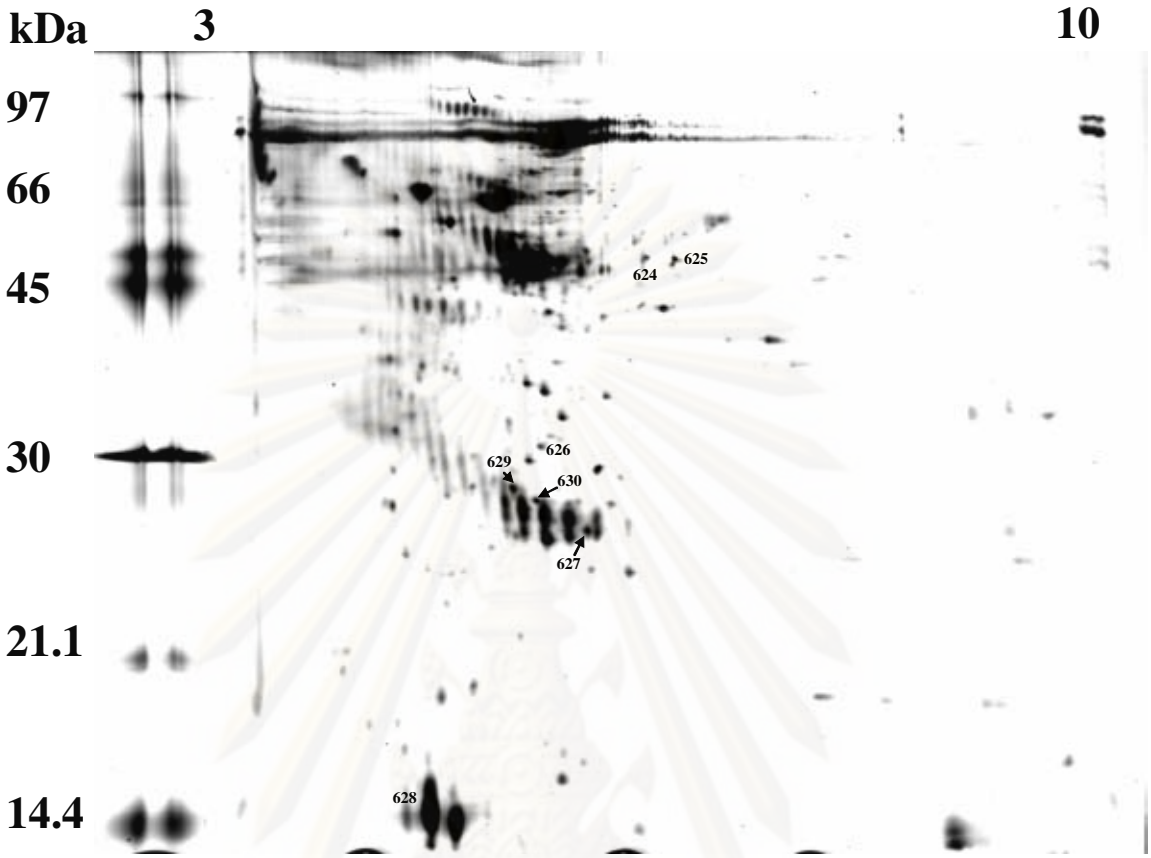


Figure 3.4 Protein profiles of testes of domesticated *P. monodon* broodstock (GSI = 0.46 and sperm sac/testis = 0.22; average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

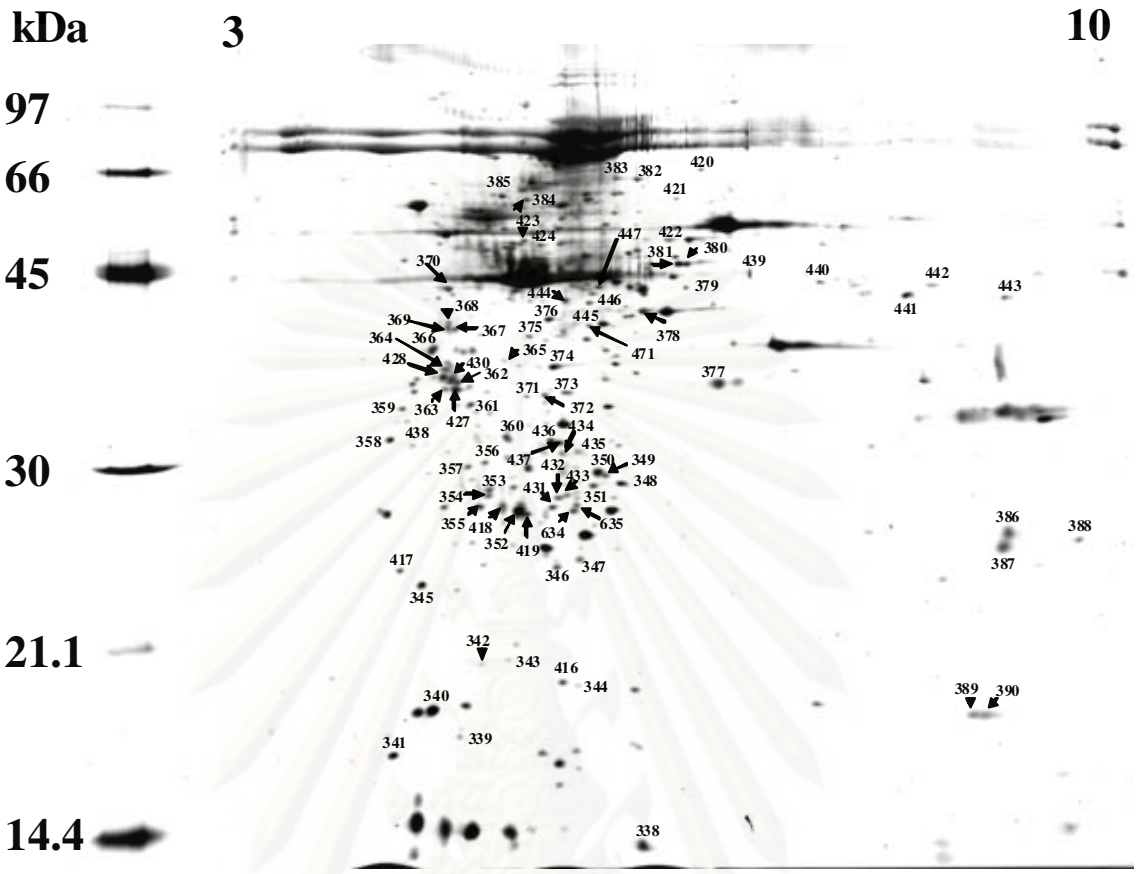


Figure 3.5 Protein profiles of testes of domesticated *P. monodon* broodstock (GSI = 0.35 and sperm sac/testis = 0.24; average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

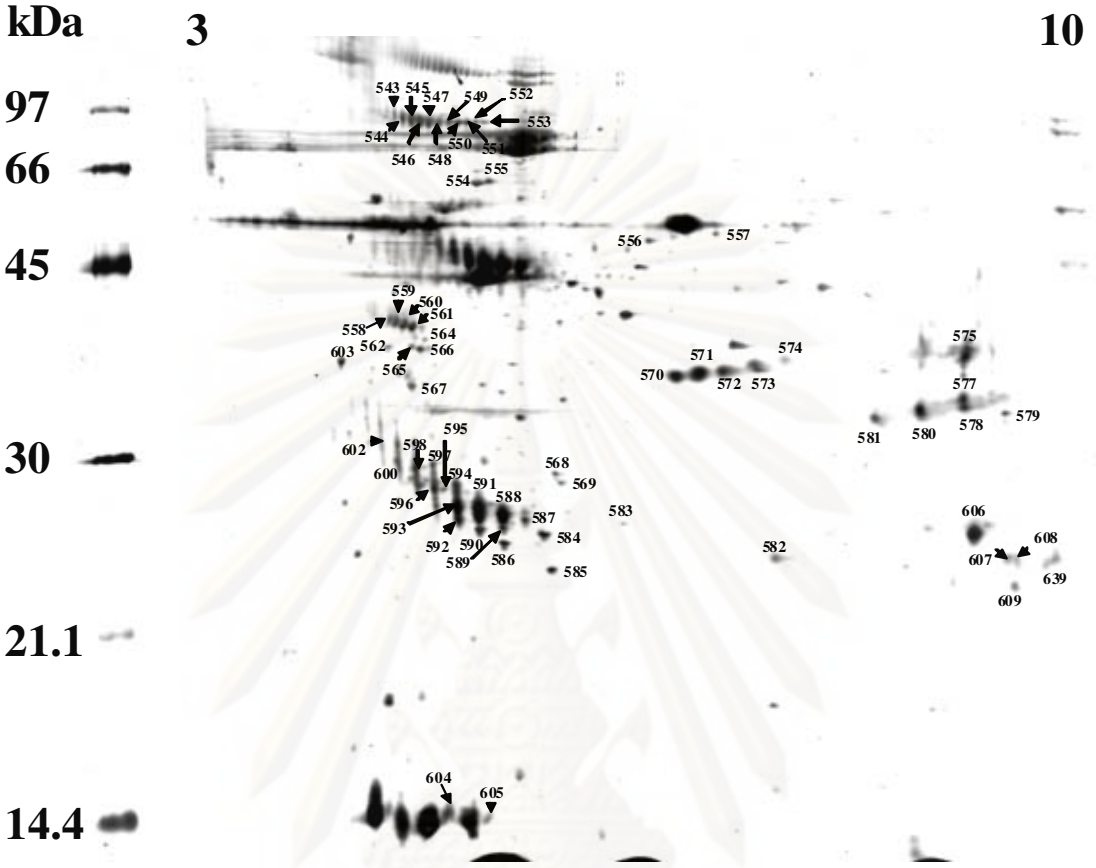


Figure 3.6 Protein profiles of testes of domesticated *P. monodon* broodstock (GSI = 0.30 and sperm sac/testis = 0.20; average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

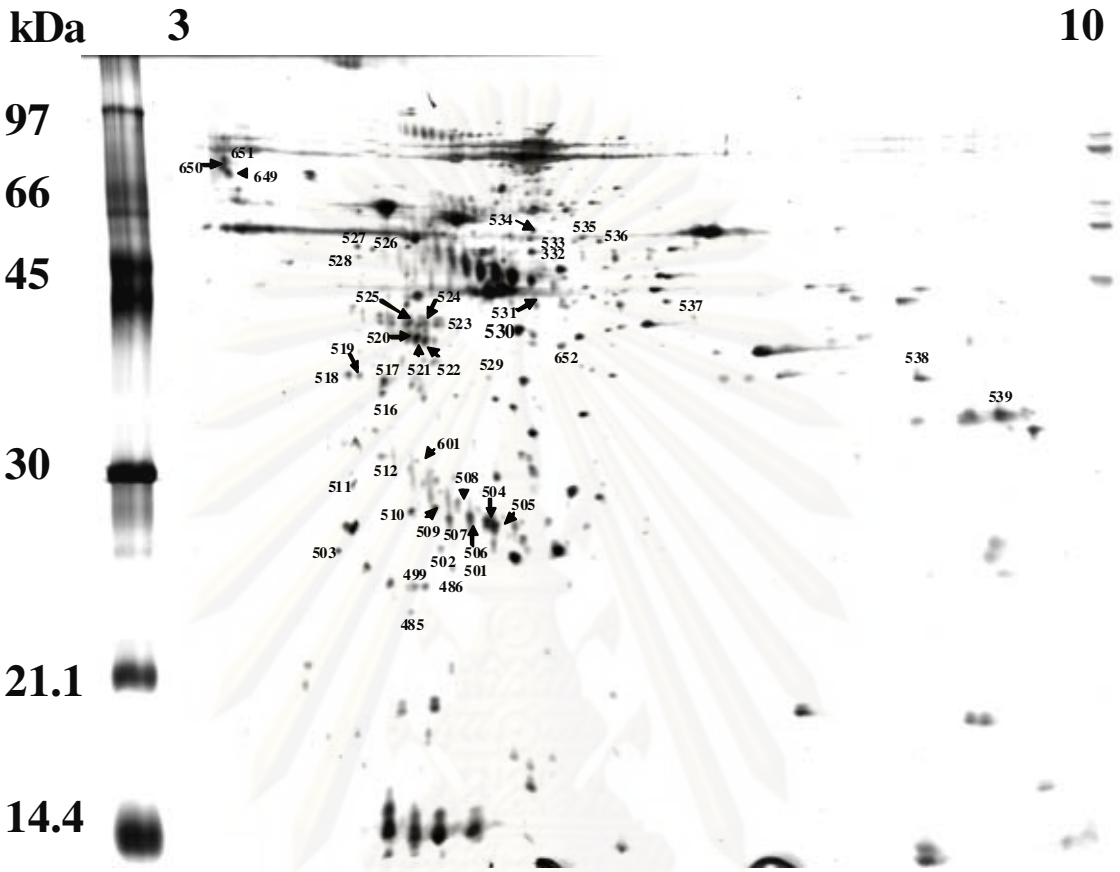


Figure 3.7 Protein profiles of of domesticated *P. monodon* broodstock (GSI = 0.29 and sperm sac/testis = 0.55; average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

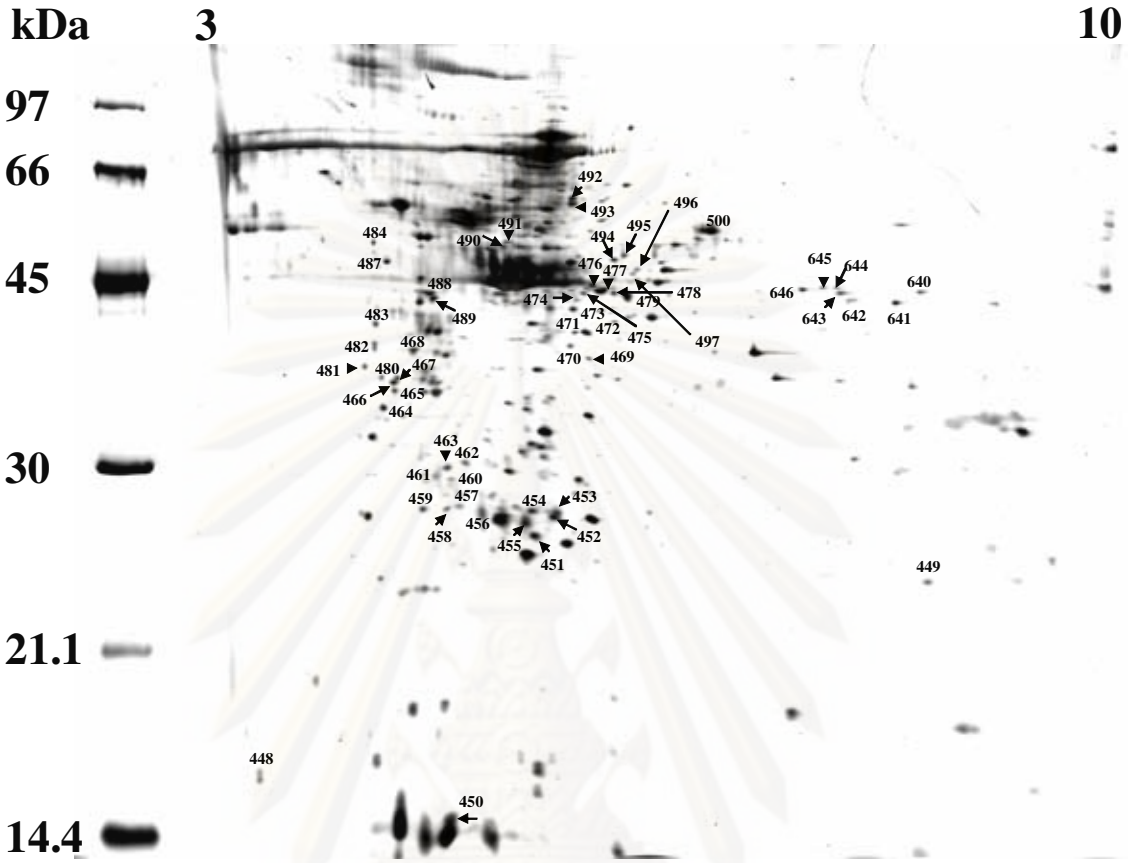


Figure 3.8. Protein profiles of testes of domesticated *P. monodon* broodstock (GSI = 0.40 and sperm sac/testis = 0.48; average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

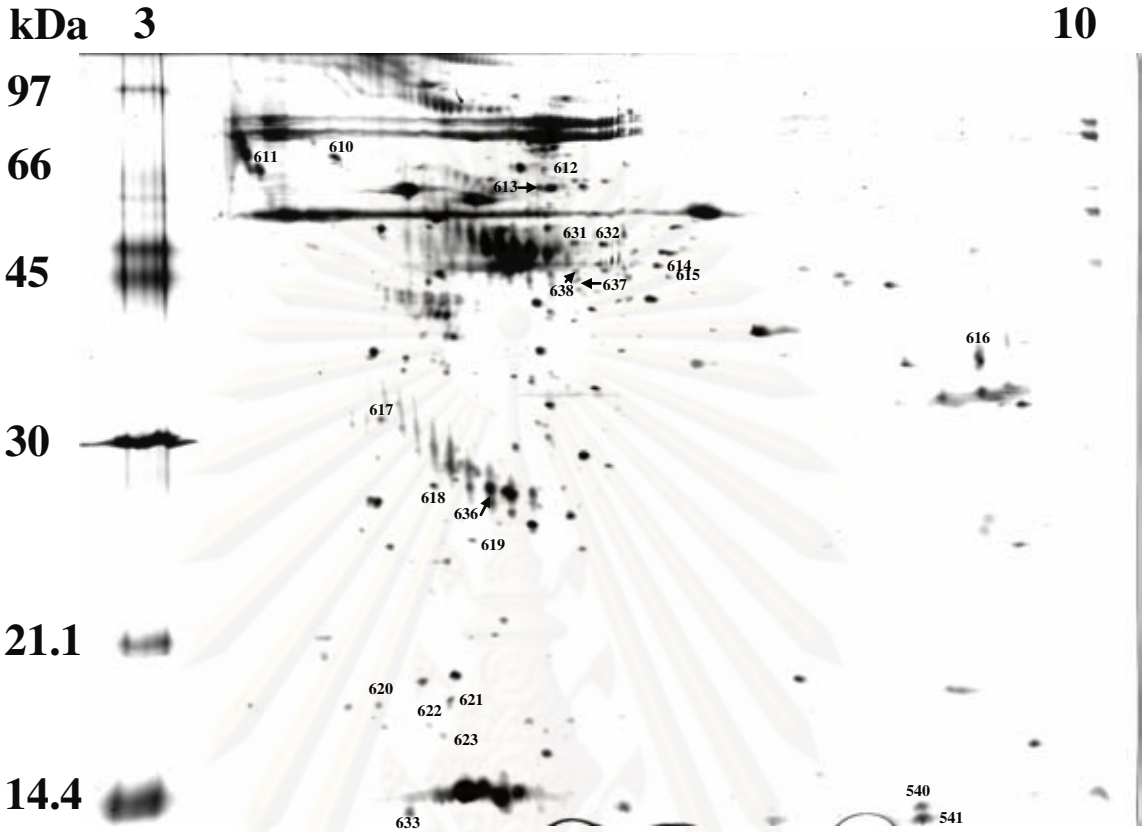


Figure 3.9 Protein profiles of testes of domesticated *P. monodon* broodstock (GSI = 0.24 and sperm sac/testis = 0.53; average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02) analyzed by two-dimensional gel electrophoresis (IEF of pH 3-10 followed by 12.5% SDS-PAGE). Numbers indicate protein spots that were identified by nanoLC-MS/MS.

3.1.2 Nano-ESI-LC-MS/MS

Total proteins extracted from wild (average GSI = 1.08 ± 0.18%, sperm sac/testis = 0.26 ± 0.06, *N* =3, group A) and domesticated broodstock (GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, group B and GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02, group C) broodstock electrophoretically fractionated by 2-DE were further characterized by nano-ESI-LC-MS/MS. Initially, 394 spots found in testicular proteins of wild broodstock were characterized by nanoLC-MS/MS. Subsequently, protein spots found in testis of domesticated broostock group B were further characterized. Most of these spots were not observed in wild broodstock (A) and some spots found in both groups A and B were also examined. Finally, protein spots found in testis of domesticated broostock group C were further characterized. Most of these spots were not observed in wild broodstock groupA and domesticated broodstock group B and some spots found in both groups A and C and in all groups of samples were also characterized.

Finally, a total of 640 protein spots were characterized including 394 spots from wild broodstock, 120 spots from domesticated broodstock group B and 126 sports from domesticated broodstock group C. Results from similarity search are illustrated by Tables 3.1-3.3, respectively.

Results from similarity search were classified to known proteins (those significantly matched known proteins in the database), unnamed proteins (those significantly matched unnamed proteins in the database), hypothetical proteins (those significantly matched hypothetical proteins in the database), unknown proteins (those significantly matched expressed sequence tags, EST in the database) and novel proteins (those did not significantly match any sequence in the database).

Novel proteins predominated in all examined groups of broodstock (200, 74 and 80 spots accounting for 50.76, 61.67 and 63.49% of examined protein spots in groups A, B and C samples, respectively). A total of 354 (55.31%) of novel proteins were found. A total of 208 spots (32.50%) significantly matched sequences in the database and considered as known proteins. The number and percentage of known proteins in respective groups of samples were 146 (37.06%), 28(23.33%) and 34 (26.98%), respectively

Table 3.1 Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
1	Unknown	HC-N-N01-2578-LF	3.0%	gil000101580	28829 /9.65	96000/5.5	62
2	Unknown	GlEp-N-N01-2148-LF	15.0%	gil000047553	28541/9.11	95000/5.9	57
3	Heat shock protein gp96	OV-N-N01-0978-W	10.0%	gil000211496	27178/4.74	100000/4.6	146
4	putative ABC transporter ATP-binding protein [*Streptomyces griseus subsp. griseus NBRC 13350*]		2.0%	gil182436389	73223/5.51	95000/4.7	58
5	Novel					93000/4.7	
6	Novel					90000/4.8	
7	Hsp-90 [*Chiromantes haematocheir*]	HC-N-N01-12368-LF	4.0%	gil000090837	27112/9.07	87000/5.00	57
8	Glycoprotein X precursor	HPa-N-N03-1190-LF	8.0%	gil000164062	24859/10.04	90000/5.00	49
9	Novel					84000/6.4	
10	Hemocyanin [*Litopenaeus vannamei*]	HPa-N-N03-0541-LF	28.0%	gil000160592	21257/5.34	80000/5.8	321
11	Hemocyanin [*Litopenaeus vannamei*]	HPa-N-N04-0536-LF	21.0%	gil000172285	27413/4.81	75000//5.8	200
12	glucose-regulated protein 78 [*Fenneropenaeus chinensis*]	OV-N-N01-0527-W	11.0%	gil000208861	26430/5.27	76000/5.0	139
13	Novel					60000/3.9	
14	Protein disulfide isomerase [*Litopenaeus vannamei*]	OV-N-S01-1324-W	13.0%	gil000223598	26256/5.42	57000/4.5	269
15	Epidermal cytokeratin 2 [*Homo sapiens*]		1.0%	gil181402	66111/ 8.07	63000/4.55	74
16	Novel					59000/4.65	
17	Novel					56000/4.7	
18	Unknown	BT-N-S01-0466-W	6.0%	gil0000007918	21082/9.61	51000/4.55	51
19	F1-ATP synthase beta subunit [*Litopenaeus vannamei*]	HC-N-N01-13801-LF	9.0%	gil000098085	25085/5.62	51000/4.65	85
20	Hypothetical protein [*Thermobia domestica*]	HPa-N-N02-0055-LF	8.0%	gil000154718	25872/9.93	54000/5.0	66
21	predicted protein [*Micromonas pusilla CCMP1545*]		3.0%	gil226458439	52600/5.76	53000/4.9	
22	Novel					50000/5.0	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
23	RecName: Full=Trypsin; Flags: Precursor		8.0%	gil136429	25078/7.00	50000/5.1	74
24	Substrate-binding transmembrane protein [*Ralstonia solanacearum GMI1000*]		1.0%	gil17544781	86235/8.45	45000/5.3	65
25	Epidermal cytokeratin 2 [Homo sapiens]		1.0%	gil181402	66111/ 8.07	45000/5.4	86
26	Novel					45000.5.6	
27	Hypothetical protein - bloodfluke planorb (fragment)	ES-N-S03-0155-W	5.0%	gil000013262	20441/9.83	43000/5.3	44
28	Chain A, Crystal Structure Of Monomeric Actin Bound To Cytochalasin D	BT-N-S01-0101-W	12.0%	gil0000006279	16659/5.01	43000/5.45	75
29	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	65000/5.5	57
30	Unknown	OV-N-N01-0669-W	7.0%	gil000209690	28769/8.70	60000/5.2	47
31	Protein-disulfide isomerase [*Scylla paramamosain*]	OV-N-S01-0764-W	13.0%	gil000220555	29075/5.60	58000/5.7	184
32	Mediator complex subunit 7 CG31390-PA isoform 1 [*Apis mellifera*]	HC-N-S01-0215-LF	6.0%	gil000142347	33043/9.30	43000/6.7	55
33	RecName: Full=Trypsin; Flags: Precursor		8.0%	gil136429	25078/7.00	44000/6.55	63
34	Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX		13.0%	gil3318722	24142/8.26	44500/6.65	134
35	Novel					45000/6.6	
36	Putative ribosomal protein L32 [*Maconellicoccus hirsutus*]	HPA-N-N01-0643-LF	19.0%	gil000153173	16322/11.72	49000/6.75	49
37	Novel					50000/7.0	
38	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	63000/6.3	49
39	Arginine kinase [*Penaeus monodon*]	GlEp-N-N01-0368-LF	11.0%	gil000037477	28024/8.72	40000/6.5	148
40	Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX		13%	gil3318722	24142 8.26	40500/6.2	106
59	Proteasome alpha 4 subunit [*Nasonia vitripennis*]	HC-N-N01-13533-LF	3.0%	gil000096890	27577/8.12	31000/5.7	49

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
60	Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX		22.0%	gi|3318722	24142/8.26	31000/5.5	170
61	Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX		13.0%	gi|3318722	24153/8.26	30000/5.4	114
62	Novel					29500/5.4	
63	Peroxiredoxin [*Penaeus monodon*]	OV-N-S01-0114-W	4.0%	gi|000216973	26185/5.81	29000/5.9	49
64	Glutathione S-transferase Mu 3 [*Anoplopoma fimbria*]	AG-N-N01-0855-W	3.0%	gi|0000003782	29371/6.12	28000/5.9	57
65	Novel					29500/6.1	
66	Glyceraldehyde-3-phosphate *dehydrogenase [Portunus* trituberculatus]	GL-H-S01-0663-LF	6.0%	gi|000021673	25737/8.31	38000/7.4	48
67	Unknown	HPO-N-S01-0350-LF	7.0%	gi|000065224	29726/9.74	36000/7.55	45
68	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	4.0%	gi|000023037	27221/6.59	26500/6.0	101
69	Proteasome delta [*Nasonia vitripennis*]	HC-N-N01-3568-LF	3.0%	gi|000106520	26398/5.38	24000/6.1	72
70	Trypsin precursor			gi|136429	25078	24000/5.5	79
71	Novel					24500/5.9	
72	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	4.0%	gi|000023037	27221/6.59	26000/5.8	84
73	Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX		13.0%	gi|3318722	24142/8.26	25000/5.6	125
74	Unknown	OV-N-N01-0669-W	7.0%	gi|000209690	28769/8.70	26500/5.5	53
75	RecName: Full=Trypsin; Flags: Precursor		8.0%	gi|136429	25078/7.00	26000/5.3	81
76	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	265005.2	52
77	PhoH-like protein [*Roseobacter phage SIO1*]		2.0%	gi|19343479	43385/9.23	27000/5.2	70
78	Novel					49000/6.0	
79	Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	HC-N-N01-12735-LF	6.0%	gi|000093024	28421/8.80	29000/4.8	103
80	Hypothetical protein TcasGA2_TC001230 [*Tribolium castaneum*]	AG-N-N01-0313-W	4.0%	gi|0000001379	28458/10.50	27000/4.5	49

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
81	Nucleoplasmin isoform 1-like protein [*Maconellicoccus hirsutus*]	GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	22000.4.5	65
82	Novel					19000/6.3	
83	RecName: Full=Trypsin; Flags: Precursor		8.0%	gil136429	25078/7.00	19000/5.7	62
84	Novel					20000/5.1	
85	RecName: Full=Trypsin; Flags: Precursor		17.0%	gil136429	25078/7.00	17500/4.8	109
86	Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX		4.0%	gil3318722	24142/8.26	16000/4.7	63
87	PhoH-like protein [*Roseobacter phage SIO1*]		2.0%	gil19343479	43385/9.23	16000/4.7	66
88	PhoH-like protein [*Roseobacter phage SIO1*]		2.0%	gil19343479	43385/9.23	15500/4.0	66
89	Zinc-containing alcohol dehydrogenase [*Dictyostelium discoideum AX4*]	BT-N-S01-0482-W	4.0%	gil0000007966	18704/9.82	24500/3.9	55
90	Unknown	ES-N-S03-0696-W	4.0%	gil000015766	27191/8.05	27000/4.1	49
91	Novel					27000/4.0	
92	Novel					27000/4.1	
93	Novel						
94	Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]		7.0%	gil123477668	20547/9.73	17500/7.8	58
95	Zinc-containing alcohol dehydrogenase [*Dictyostelium discoideum AX4*]	BT-N-S01-0482-W	4.0%	gil0000007966	18704/9.82	31000/4.15	47
96	Novel					31000/4.05	
97	Novel					32000/4.9	
98	Trypsin precursor				25078	17000/4.5	62
99	Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]		7.0%	gil123477668	20547/9.73	45000/5.15	64
100	Novel					45000/5.25	
101	Receptor for activated protein kinase c1 [*Penaeus monodon*]	AG-N-N01-0283-W	4.0%	gil0000001237	27631/6.25	37000/8.7	63

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
102	Novel					32000/9.3	
103	Voltage-dependent anion-selective channel isoform 1 [*Tribolium castaneum*]	AG-N-N01-1147-W	6.0%	gil0000005006	20977/9.06	32000/9.5	52
104	Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX			gil3318722	24142	35000/4.3	135
105	Novel					34500/4.25	
106	Trypsin precursor			gil136429	25078	32000/4.5	60
107	Novel					30000/4.7	
108	Type II keratin subunit protein			gil386854	52928	29000/4.8	71
109	Novel					28000/5.1	
110	Peroxiredoxin [Fenneropenaeus indicus]	HC-H-S01-0335-LF	7.0%	gil000070688	23660/5.49	27500/4.7	70
111	Novel					27000/6.5	
112	Novel					30000/8.25	
113	Novel					25000/8.25	
114	Cyclophilin A [Penaeus monodon]	BT-N-S01-0099-W	12.0%	gil0000006266	20692/8.70	17500/9.1	142
115	Novel					15500/5.5	
116	Intracellular fatty acid binding protein [*Penaeus monodon*]	ES-N-S01-0117-W	5.0%	gil0000009405	26559/8.92	15000/5.7	107
117	Novel					15500/5.9	
118	Adenosine kinase 2 [*Culex quinquefasciatus*]	HPA-N-N01-0792-LF	3.0%	gil000153845	24514/9.72	40500/5.5	54
119	Novel					40000/5.7	
120	Novel					44000/6.1	
121	Novel					45000/6.4	
122	Novel					58000/6.1	
123	Novel					62000/6.1	
124	Novel					62000/6.15	
125	Novel					60000/5.9	
126	Novel					66000/6.85	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
127	Chaperonin containing TCP1, subunit 6A (zeta 1), isoform CRA b [*Homo sapiens*]	HC-W-S01-0248-LF	11.0%	gil000148503	12383/9.36	59000/7.1	91
128	Novel					58000/6.55	
129	Novel					42000/8.5	
130	Novel					42000/6.9	
131	Novel					38000/7.1	
132	Novel					40500/6.9	
133	Novel					43000/7.8	
134	Novel					44500/8.0	
135	Novel					43000/7.8	
136	Novel					42500/8.1	
137	Novel					970007.05	
138	Novel					42500/5.75	
139	Novel					420005.9	
140	Novel					42000/5.3	
141	Novel					54000/6.1	
142	Novel					47000/6.4	
143	Novel					22500/5.9	
144	Novel					16000/6.2	
145	Novel					32500/4.3	
146	Novel					38000/6.6	
147	Novel					35000/5.0	
148	Novel					36000/5.2	
149	Novel					44000/6.7	
150	Novel					39000/5.9	
151	Novel					50000/6.0	
152	Novel					39000/5.5	
153	Novel					39000/5.4	
154	Novel					27000/4.9	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
157	Novel					16500/3.1	
158	Novel					54000/8.1	
159	Novel					49000/8.2	
160	Novel					45000/7.9	
161	Novel					53000/7.9	
162	Glyceraldehyde 3-phosphate dehydrogenase [*Cambarus hamulatus*]	GL-H-S01-0820-LF	12.0%	gi|000022387	25544/6.38	39000/7.2	124
163	Novel					38000/7.0	
164	Unnamed protein product [*Homo sapiens*]		1.0%	gi|28317	59720/5.17	36000/6.65	64
165	Tumor necrosis factor superfamily, member 5-induced protein 1	HC-N-N01-3133-LF	12.0%	gi|000104189	18123/9.89	90000/6.15	53
166	Novel					60000/6.15	
167	Arginine kinase [*Penaeus monodon*]	AG-N-N01-1003-W	7.0%	gi|0000004411	28557/7.83	41000/6.3	68
168	Novel					50000/6.8	
169	Novel					34500/5.8	
170	Novel					33000/5.45	
171	Novel					25000/5.3	
172	Novel					26000/5.6	
173	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	4.0%	gi|000023037	27221/6.59	26500/5.9	78
174	Expressed protein [*Arabidopsis thaliana*]	LP-Y-S01-0572-LF	6.0%	gi|000203797	20943/9.59	29000/5.7	46
175	Novel					18000/4.3	
176	p23-like protein [*Apis mellifera*]	HC-H-S01-0086-LF	8.0%	gi|000069261	20782/12.00	18000/4.2	47
177	Unnamed protein product [*Homo sapiens*]		1.0%	gi|28317	59720/5.17	18000/7.4	56
178	p23-like protein [*Apis mellifera*]	HC-H-S01-0086-LF	8.0%	gi|000069261	20782/12.00	39000/4.7	47
179	Novel					44000/6.4	
180	Novel					44500/6.4	
181	Novel					41000/4.7	
182	Novel					41000/4.6	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
183	Novel					41500/6.15	
184	Novel					40000/6.1	
185	PhoH-like protein [*Roseophage SIO1*]		2.0%	gil19343479	43385/9.23	41000/5.8	58
186	Novel					29000/5.2	
187	Novel					37000/5.4	
188	Trypsin precursor				25078	41500.5.4	76
189	Novel					18000/4.5	
190	Novel					90000/5.35	
191	Novel					66000/3.1	
192	Y43E12A.2	ES-N-S03-0713-W	5.0%	gil000015867	27367/9.70	39000/5.9	60
193	Novel					44000/4.6	
194	Novel					42000/4.5	
195	Novel					42500/4.6	
196	Novel					26000/5.3	
197	Unnamed protein product [*Homo sapiens*]		3.0%	gil28317	59720/5.17	26000/5.1	131
198	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gil000070688	23660/5.49	27000/5.0	57
199	Novel					27000/4.9	
200	hypothetical protein BradDRAFT_3909 [*Bradyrhizobium sp. BTAi1*]		3.0%	gil78696479	20528/7.88	28000/4.8	60
201	Novel					90000/5.0	
202	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	90000/5.1	46
203	hypothetical protein Nwi_0969 [*Nitrobacter winogradskyi Nb-255*]		5.0%	gil75675162	27280/5.28	90000/5.2	62
204	Novel				22080/5.98	90000/5.3	
205	Hemocyanin [*Litopenaeus vannamei*]	HPa-N-N03-0671-LF	5.0%	gil000161293	22080/5.98	80000/5.35	54
206	Heat shock protein 60 [*Litopenaeus vannamei*]	OV-N-N01-0358-W	4.0%	gil000207891	26264/9.34	60000/5.0	77
207	Protein-disulfide isomerase [*Scylla paramamosain*]	OV-N-N01-0752-W	10.0%	gil000210181	24060/5.20	58000/5.5	132
208	Novel					48000/5.5	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
209	Nucleotidase [*Pseudoalteromonas tunicata D2*]		4.0%	gi|88859896	25095/4.79	48000/5.6	55
210	Adenosylhomocysteinase A [*Xenopus laevis*]	OV-N-N01-0647-W	6.0%	gi|000209564	24676/5.98	45000/5.7	61
211	Novel					48000/5.85	
212	Novel					44500/5.7	
213	Adenosylhomocysteinase [*Strongylocentrotus purpuratus*]	HPa-N-N03-1440-LF	11.0%	gi|000165447	26692/5.33	45000/5.9	137
214	Protease, serine, 1 [*Mus musculus*]		8.0%	gi|16716569	26802/4.75	44500/6.0	104
215	Unknown	HC-H-S01-0193-LF	5.0%	gi|000069873	23555/10.35	47000/6.1	50
216	Novel					47000/6.15	
217	Novel					19500/5.0	
218	Novel					18500/5.9	
219	Intracellular fatty acid binding protein [*Penaeus monodon*]	LP-N-N01-0788-LF	4.0%	gi|000192873	21988/7.75	15500/5.3	52
220	Cytochrome b [*Litopenaeus stylirostris*]	HC-N-N01-3594-LF	6.0%	gi|000106676	29347/9.26	14.700/5.3	46
221	Unknown	ES-N-S03-0550-W	4.0%	gi|000015050	23237/10.29	14400/4.1	51
222	Chain A, Crystal Structure of Putative Holliday Junction Resolvase		6.0%	gi|40889964	16412/6.07	46000/6.8	58
223	Novel					44700/6.4	
224	Novel					45000/6.4	
225	Novel					44900/6.6	
227	Novel					45000/6.6	
228	Wdtc1 protein *[Mus musculus*]		3.0%	gi|22028134	39988/5.55	36000/4.2	58
229	Novel					36000/4.1	
230	Novel					31000/3.9	
231	Novel					45000/7.15	
232	Histone protein Hist2h3c1 [*Monodelphis domestica*]	ES-N-S03-0309-W	5.0%	gi|000013917	18784/11.27	44000/7.45	61

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
233	Polarized growth protein [*Aspergillus fumigatus Af293*]		1.0%	gil7098924	109535/8.37	43000/7.25	60
334	Unknown	BT-N-S01-0251-W	6.0%	gil0000006915	19989/8.33	44900/5.45	50
235	Unknown	AG-N-N01-0248-W	21.0%	gil0000001059	6634/5.52	45000/5.3	62
236	Novel					45000/5.2	
237	Predicted protein [*Nematostella vectensis*]	ES-N-S03-0230-W	6.0%	gil000013568	16297/12.07	46000/5.1	56
238	ATP binding / kinase/ protein serine/threonine kinase [*Arabidopsis thaliana*]		1.0%	gil15226197	79284/5.75	48000/4.95	60
239	Novel					52000/4.8	
240	Histone protein Hist2h3c1 [*Monodelphis domestica*]	ES-N-S03-0309-W	5.0%	gil000013917	18784/11.27	49000/7.1	56
241	Novel					49000/7.8	
242	40S ribosomal protein S2	OV-N-ST02-0027-LF	10.0%	gil000231553	109147.16	49000/8.0	44
243	Predicted protein [*Nematostella vectensis*]	ES-N-S03-0230-W	6.0%	gil000013568	16297/12.07	54000/8.1	53
244	Novel					48000/8.15	
245	Novel					14000/4.1	
246	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	3.0%	gil000232640	26299/9.57	14400/4.1	65
247	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	3.0%	gil000232640	26299/9.57	15500/4.1	59
248	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	3.0%	gil000232640	26299/9.57	144000/4..4	68
249	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	3.0%	gil000232640	26299/9.57	14400/4.7	56
250	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	3.0%	gil000232640	26299/9.57	14400/5.2	73
251	Novel					17500/5.1	
252	Novel					17000/5.1	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
253	Novel					162005.4	
254	Cyclophilin A [Penaeus monodon]	BT-N-S01-0099-W	7.0%	gil0000006266	20692/8.70	14000/6.1	80
255	Ribosomal protein P2 [*Strongylocentrotus purpuratus*]	ES-N-S03-1095-W	48.0%	gil000017899	14865/5.97	18500/3.7	388
256	Novel					21000/3.5	
257	Novel					21500/3.6	
258	Novel					16200/7.4	
259	Hypothetical protein TM1040_2050 [*Silicibacter sp. TM1040*]		7.0%	gil99081890	12396/10.09	16000/7.9	59
260	rRbulose-1,5-bisphophate carboxylase/oxygenase small subunit [*Vitis pseudoreticulata*]		7.0%	gil86156014	20671/9.06	16000/8.4	97
261	Cyclophilin A [*Penaeus monodon*]	BT-N-S01-0099-W	26.0%	gil0000006266	20692/8.70	19000/8.7	148
262	Novel					19000/8.6	
263	Novel					26000/3.8	
264	Sarcoplasmic calcium-binding protein [*Litopenaeus vannamei*]	AG-N-N01-0210-W	11.0%	gil000000897	27761/5.55	25000/4.2	106
265	Nucleoplasmin isoform 1-like protein [*Maconellicoccus hirsutus*]	GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	24500/4.6	67
266	Nucleoplasmin isoform 1-like protein [*Maconellicoccus hirsutus*]	GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	24500/4.5	67
267	Novel					24500/4.2	
268	Novel					28100/4.0	
269	Novel					28500/3.9	
270	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gil000070688	23660/5.49	28200/4.6	52
271	Novel					27500/4.8	
272	Novel					25500/4.8	
273	Novel					26000/5.0	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
274	Novel					27050/5.5	
275	Unnamed protein product [*Homo sapiens*]		2.0%	gi|28317	59720/5.17	27500/5.45	86
276	Novel					27000/5.0	
277	ATP synthase F0 subunit 6 [*Penaeus monodon*]	HPO-N-S01-0024-LF	5.0%	gi|000063499	26914/9.44	27500/54.8	48
278	Novel					27500/5.3	
279	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	4.0%	gi|000023037	27221/6.59	28000/5.3	93
280	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	9.0%	gi|000023037	27221/6.59	28500/5.0	134
281	Novel					30000/4.9	
282	Unknown	OV-N-N01-0669-W	7.0%	gi|000209690	28769/8.70	32200/4.0	61
283	Cytosolic malate dehydrogenase thermolabile form [*Sphyraena idiastes*]		5.0%	gi|14583131	36463/6.60	40000/5.8	60
284	Cytosolic manganese superoxide dismutase [*Penaeus monodon*]	GlEp-N-S01-1341-LF	8.0%	gi|000059095	25091/5.57	33000/5.0	97
285	Cytosolic manganese superoxide dismutase [*Penaeus monodon*]	GlEp-N-S01-1341-LF	14.0%	gi|000059095	25091/5.57	34000/4.9	187
286	Electron-transfer-flavoprotein, alpha polypeptide [*Danio rerio*]	HC-N-N01-0997-LF	4.0%	gi|000079053	27958/8.41	34500/4.9	74
287	Putative acidic p0 ribosomal protein [*Toxoptera citricida*]	GL-H-S01-0619-LF	9.0%	gi|000021439	26882/9.07	37000/4.9	136
288	Cytosolic manganese superoxide dismutase [*Penaeus monodon*]	GlEp-N-S01-1341-LF	20.0%	gi|000059095	25091/5.57	35000/5.1	244
289	Arginine kinase [*Penaeus monodon*]	GlEp-N-N01-0368-LF	11.0%	gi|000037477	28024/8.72	41000/6.1	152
291	hypothetical protein BRAFLDRAFT_79044 [*Branchiostoma floridae*]	AG-N-N01-0546-W	5.0%	gi|0000002391	26985/9.35	41000/4.9	97
293	Hypothetical protein BRAFLDRAFT_79044 [*Branchiostoma floridae*]	HC-N-N01-1115-LF	6.0%	gi|000085199	27890/7.56	31500/6.0	63

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
294	Calreticulin precursor [*Fenneropenaeus chinensis*]	IN-N-S01-0553-LF	15.0%	gil000185623	21676/5.55	63000/3.7	144
295	Calreticulin precursor [*Fenneropenaeus chinensis*]	GlEp-N-S01-1589-LF	14.0%	gil000060373	16288/5.58	60000/3.8	94
296	Hypothetical protein BRAFLDRAFT_115608 [*Branchiostoma floridae*]	OV-N-S01-1780-W	23.0%	gil000225962	27909/8.29	44000/6.2	184
297	Eukaryotic translation initiation factor 3 subunit E (Eukaryotic translation initiation factor 3 subunit 6) (eIF-3 p48) (eIF3e) (Viral integration site protein INT-6 homolog) [*Sus scrofa*]	AG-N-N01-0474-W	4.0%	gil0000002106	25099/8.95	57000/4.8	85
298	Novel					55000/5.0	
299	Unnamed protein product [*Paramecium tetraurelia*]		3.0%	gil124424210	41032/4.75	55000/5.0	71
300	Unknown	HC-N-N01-8453-LF	19.0%	gil000133797	6571/11.00	55000/5.1	76
301	Unnamed protein product [*Paramecium tetraurelia*]		3.0%	gil124424210	41032/4.75	55000/5.2	67
302	Hypothetical protein BRAFLDRAFT_114917 [*Branchiostoma floridae*]	AG-N-N01-0407-W	5.0%	gil0000001778	26755/6.66	53000/5.5	101
303	70 kD heat shock protein [*Mirocaris fortunata*]	AG-N-N01-0802-W	5.0%	gil0000003572	26393/5.74	64000/4.9	63
304	Unknown	TT-N-S01-0497-W	7.0%	gil000235039	23572/6.64	4400/4.95	112
305	Novel					45000/6.0	
306	Novel					58000/5.25	
307	Unnamed protein product [*Homo sapiens*]		2.0%	gil28317	59720/5.17	56000/5.7	89
308	Novel					60000/5.6	
309	NADP-dependent leukotriene B4 12-hydroxydehydrogenase [*Gallus gallus*]	OV-N-N01-0347-W	4.0%	gil000207823	26428/7.11	42000/4.9	72

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
310	Furin-like protease 1, isoforms 1/1-X/2 precursor (Furin-1) (Kex2-like endoprote		3.0%	gil91092736	88995/7.61	87000/4.75	135
311	Methylmalonate-semialdehyde dehydrogenase [*Aedes aegypti*]	GL-H-S01-1029-LF	7.0%	gil000023126	26676/6.51	53000/5.8	65
312	Novel					58000/5.9	
313	Hypothetical protein Nham_3970 [*Nitrobacter hamburgensis X14*]		5.0%	gil92119376	19666/9.92	55000/5.9	70
314	Novel					59000/6.1	
315	Hypothetical protein BRAFLDRAFT_119287 [*Branchiostoma floridae*]	AG-N-N01-0134-W	4.0%	gil000000541	27356/6.95	42000/6.4	58
316	ENSANGP00000020121 [*Anopheles gambiae str. PEST*]	HC-H-S01-0530-LF	4.0%	gil000071804	27016/9.02	39300/5.4	59
317	Hypothetical protein LOC553452 [*Danio rerio*]	HC-N-S01-0103-LF	3.0%	gil000141719	28487/8.94	39000/5.4	66
318	Novel					35500/3.9	
319	Novel					46000/4.0	
320	Novel					45000/3.5	
321	Voltage-dependent anion-selective channel isoform 1 [*Tribolium castaneum*]	HPa-N-N03-1685-LF	21.0%	gil000166545	26037/8.99	31000/9.3	216
322	Novel					332000/9.0	
323	Novel					33000/9.0	
324	Glyceraldehyde-3-phosphate dehydrogenase [*Portunus trituberculatus*]	GL-H-S01-0663-LF	16.0%	gil000021673	25737/8.31	38000/6.9	170
325	Novel					42000/8.3	
326	Fructose 1,6-bisphosphate aldolase [*Artemia franciscana*]	AG-N-N01-0522-W	6.0%	gil0000002294	27166/8.61	42500/7.85	54
327	Novel					36000/6.0	
328	Novel					25000/9.2	

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
329	Novel					260009.6	
330	Novel					27000/9.0	
331	Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	TT-N-ST01-0056-W	14.0%	gil000238206	26814/9.14	29800/4.6	183
332	Novel					27500/4.4	
333	Novel					32500/8.6	
334	Novel					32000/8.6	
335	Novel					35000/6.6	
336	Novel					49000/4.4	
337	Novel					96000/4.5	
391	Hypothetical protein CBG09936 [*Caenorhabditis briggsae*]		1.0%	gil39590708	96606/6.16	97000/5.1	90
392	Hypothetical protein TcasGA2_TC001048 [*Tribolium castaneum*]	HT-N-S01-0023-LF	9.0%	gil000061736	16159/4.93	19500/4.35	51
393	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	19500/4.7	52
394	Protein-disulfide isomerase [*Scylla paramamosain*]	OV-N-N01-0752-W	24.0%	gil000210181	24060/5.20		258
395	Intracellular fatty acid binding protein [*Penaeus monodon*]	LP-N-N01-0788-LF	11.0%	gil000192873	21988/7.75	17000/5.0	141
396	40S ribosomal protein [*Perinereis aibuhitensis*]	AG-N-N01-0667-W	11.0%	gil0000002947	21176/8.41	17500/5.3	119
397	Eukaryotic initiation factor 4A [*Callinectes sapidus*]	AG-N-N01-0171-W	5.0%	gil000000711	26870/4.83	49000/4.85	55
398	Eukaryotic initiation factor 4A [*Callinectes sapidus*]	AG-N-N01-0171-W	5.0%	gil000000711	26870/ 4.83	46000/4.95	58
399	Unknown	AG-N-N01-0248-W	21.0%	gil0000001059	6634/5.52	44500/5.01	85
400	Actin 2 [*Penaeus monodon*]	AG-N-N01-0779-W	3.0%	gil0000003476	26914/4.85	44500/4.7	49
401	Unknown	AG-N-N01-0248-W	21.0%	gil0000001059	6634/5.52	44000/5.35	79

Table 3.1 (cont.) Characterized protein spots in testes of wild *P. monodon* broodstock (group A) (average GSI = 1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06, *N* =3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
402	GDP dissociation inhibitor		4.0%	gil157492	51144/7.57	48000/5.0	84
403	Novel					44000/5.1	
404	Novel					43800/5.9	
405	Novel					43000/6.1	
406	Novel					44800/6.0	
407	Alcohol dehydrogenase [*Bombyx mori*]	GL-N-STC02-0149-LF	7.0%	gil000030547	17936/5.73	44000/6.35	48
408	Rh type B glycoprotein [*Hylobates sp*.]		1.0%	gil17223572	49366/6.35	38500/5.7	63
409	Malate dehydrogenase (EC 1.1.1.37), mitochondrial – pig		7.0%	gil65932	33518/8.55	36500/7.3	95
410	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gil000070688	23660/5.49	27300/5.2	57
411	Putative oncoprotein nm23 [*Ictalurus punctatus*]		19.0%	gil000080101	23317/8.76	19500/7.5	239
412	Novel					17800/4	
413	Unnamed protein product [*Homo sapiens*]		1.0%	gil28317	59720/5.17	27300/5.1	67
414	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	21.0%	gil000070688	23660/5.49	27200/5.05	202

Table 3.2 Characterized protein spots in testes of domesticated *P. monodon* broodstock (group B), (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
338	Novel					14400/6.3	
339	Novel					18000/4.8	
340	Novel					19000/4.6	
341	Novel					17300/4.2	
342	Novel					19500/5.0	
343	Novel					19600/5.25	
344	Novel					198000/5.2	
345	Novel					24500/4.45	
346	Novel					25000/5.5	
347	Novel					255000/5.8	
348	Triosephosphate isomerase [*Fenneropenaeus chinensis*]	HC-N-N01-12801-LF	6.0%	gil000093418	27631/9.42	28000/6.0	77
349	Novel					29500/5.9	
350	Novel					29500/5.9	
351	Novel					28000/5.95	
352	Novel					28000/5.35	
353	Novel					28500/5.1	
354	Novel					28500/5.0	
355	Novel					29000/5.0	
356	Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	HC-N-N01-12735-LF	6.0%	gil000093024	28421/8.80	300000/5.0	111
357	Novel					30000/4.9	
358	Novel					32000/4.4	
359	Novel					34500/4.4	
360	Novel					32000/5.2	
361	Novel					35000/9.0	
362	Novel					35500/5.01	

Table 3.2 (cont.) Characterized protein spots in testes of domesticated *P. monodon* broodstock (group B), (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
363	Novel					35500/4.7	
364	Novel					37500/4.7	
365	Novel					38500/5.2	
366	Novel					38000/4.6	
367	Novel					40000/4.8	
368	Novel					40000/4.7	
369	Novel					40000/4.65	
370	Laminin receptor [*Litopenaeus vannamei*]	HC-W-S01-0862-LF	12.0%	gil000150266	19503/9.04	44000/4.8	121
371	Novel					35000/5.3	
372	Novel					35000/5.5	
373	Novel					35000/5.7	
374	Putative acidic p0 ribosomal protein [*Toxoptera citricida*]	GL-H-S01-0619-LF	8.0%	gil000021439	26882/9.07	38000/5.6	134
375	Pancreatic trypsin 1 [*Rattus norvegicus*]			gil6981420	26627	40000/5.4	63
376	Novel					41000/5.5	
377	Novel					36000/6.7	
378	Arginine kinase [*Penaeus monodon*]	GlEp-N-N01-0368-LF	11.0%	gil000037477	28024/8.72	41000/6.25	93
379	Hypothetical protein BRAFLDRAFT_119287 [*Branchiostoma floridae*]	AG-N-N01-0134-W	4.0%	gil000000541	273566.95	43500/6.5	58
380	Novel					45000/6.55	
381	Novel					45000/6.45	
382	Novel					65000/6.2	
383	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	65000/6.1	46
384	Novel					55000/5.45	
385	Heat shock protein 60 [*Litopenaeus vannamei*]	TT-N-S01-0846-W	8.0%	gil000236557	26532/5.11	57000/5.3	132
386	Novel					2000/9.2	
387	Novel					26500/9.15	
338	Novel					26500/9.8	

Table 3.2 (cont.) Characterized protein spots in testes of domesticated *P. monodon* broodstock (group B), (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
389	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	19000/8.8	48
390	Cyclophilin A [*Penaeus monodon*]	BT-N-S01-0099-W	31.0%	gil0000006266	20692/8.70	19000/9.0	211
416	GJ21900 [*Drosophila virilis*]	HC-H-S01-0582-LF	26.0%	gil000072104	27806/7.25	20000/5.65	298
417	Novel					25000/4.2	
418	Unnamed protein product [*Homo sapiens*]		6.0%	gil28317	59720/5.17	28000/5.2	255
419	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gil000070688	23660/5.49	27500/5.3	58
420	Novel					66000/6.55	
421	Novel					55000/6.5	
422	Novel					48500/6.45	
423	Novel					48000/5.5	
424	Novel					46000/5.5	
425	Novel					38500/5.0	
426	Novel					38500/4.85	
428	Hemocyanin [*Litopenaeus vannamei*]	HPa-N-N03-0541-LF	12.0%	gil000160592	21257/5.34	37000/4.7	103
429	Novel					37000/5.0	
430	Novel					37000/4.8	
431	Unnamed protein product [*Homo sapiens*]		5.0%	gil28317	59720/5.17	28000/5.5	218
432	Hemocyanin [*Litopenaeus vannamei*]	HPA-N-N01-0022-LF	10.0%	gil000150392	10473/6.45	28500/5.6	60
433	Novel					29000/5.6	
434	Novel					30000/5.7	
435	Hemocyanin [*Litopenaeus vannamei*]	HPa-N-N03-0541-LF	16.0%	gil000160592	21257/5.34	31000/5.8	150
436	Proteasome subunit alpha type [*Aedes aegypti*]	LP-N-N01-0262-LF	10.0%	gil000190425	20323/6.06	32000/5.6	121
437	Unnamed protein product [*Homo sapiens*]		1.0%	gil28317	59720/5.17	32000/5.65	70
438	14-3-3-like protein [*Penaeus monodon*]	AG-N-N01-0324-W	9.0%	gil0000001429	27959/5.06	32000/4.4	155
439	Novel					44500/7.0	

Table 3.2 (cont.) Characterized protein spots in testes of domesticated *P. monodon* broodstock (group B), (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
440	Hypothetical protein TcasGA2_TC014998 [*Tribolium castaneum*]	HT-N-S01-0297-LF	14.0%	gi|000062817	11120/6.08	44000/7.6	98
441	Aldolase [*Branchiostoma belcheri*]		3.0%	gi|2244609	38811/8.11	43000/8.3	68
442	GK22671 [*Drosophila willistoni*]	TT-N-S01-0178-W	14.0%	gi|000233415	17146/7.79	44000/8.55	154
443	Novel					43000/9.2	
444	Novel					43000/5.7	
445	Adenosine kinase 2 [*Culex quinquefasciatus*]	HPA-N-N01-0792-LF	3.0%	gi|000153845	24514/9.72	43000/5.9	49
446	Novel					43000/5.9	
447	Novel					44000/6.0	
543	Novel					97000/4.5	
544	Novel					96000/4.6	
545	Novel					96000/4.65	
546	Novel					960000/4.7	
547	Novel					90000/4.8	
548	Novel					90000/4.9	
549	Novel					90000/5.0	
550	Novel					90000/5.05	
551	Novel					87000/5.15	
552	Novel					87000/5.2	
553	Novel					87000/5.25	
554	Novel					63000/5.2	
555	Novel					63500/5.3	
556	Novel					49000/6.4	
557	Novel					53000/7.15	
558	Novel					41500/4.45	
559	Novel					40500/4.5	
560	Novel					40500/4.6	
561	Novel					41000/4.65	

Table 3.2 (cont.) Characterized protein spots in testes of domesticated *P. monodon* broodstock (group B), (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
562	Novel					38500/4.4	
563	Novel					37500/4.0	
564	Novel					39500/4.7	
565	Novel					38500/4.6	
566	Unknown	HC-N-N01-14005-LF	5.0%	gil000099077	20089/11.23	38500/4.65	56
567	Novel					35000/4.6	
568	Novel					29000/5.75	
569	Triosephosphate isomerase [*Fenneropenaeus chinensis*]	HC-N-N01-7864-LF	33.0%	gil000130550	25907/6.58	28500/5.8	243
570	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	36500/6.7	75
571	Novel					36500/6.9	
572	Novel					37500/7.2	
573	Novel					37500/7.4	
574	Novel					38000/7.7	
575	Novel					42000/9.2	
576	Unknown	HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	39000/9.2	48
577	Novel					38000/9.2	
578	Envelope glycoprotein [*Human immunodeficiency virus 1*]		3.0%	gil35396864	48265/9.33	39000/9.2	69
579	Hypothetical protein TcasGA2_TC006408 [*Tribolium castaneum*]	AG-N-N01-0719-W	9.0%	gil0000003182	11090/7.85	36000/9.6	49
580	Novel					37000/8.8	
581	Novel					35000/8.4	
582	Novel					25500/7.7	
583	Novel					28000/6.4	
584	Novel					27000/5.7	
585	Novel					25000/5.8	
586	Novel					26500/5.3	
587	Novel					27500/5.5	

Table 3.2 (cont.) Characterized protein spots in testes of domesticated *P. monodon* broodstock (group B), (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
589	Novel					27000/5.4	
590	Novel					26500/5.2	
591	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gil000070688	23660/5.49	27500/5.2	61
592	Unknown	OV-N-N01-0669-W	7.0%	gil000209690	28769/8.70	265000/5	48
593	Novel					27500/5.0	
594	Novel					29000/5.0	
595	Novel					28500/4.8	
596	Unknown	OV-N-N01-0056-W	2.0%	gil000206110	26565/8.98	28500/4.9	52
597	Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	HC-N-N01-12735-LF	6.0%	gil000093024	28421/8.80	30000/4.8	104
598	Novel					30000/4.7	
599	14-3-3-like protein [*Penaeus monodon*]	AG-N-N01-0324-W	4.0%	gil0000001429	27959/5.06	32000/4.7	63
600	Novel					30000/4.45	
602	Novel					31000/4.4	
603	Sarcoplasmic calcium-binding protein [*Litopenaeus vannamei*]	AG-N-N01-0210-W	9.0%	gil000000897	27761/5.55	25500/4.2	151
604	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	8.0%	gil000232640	26299/9.57	14500/5.0	120
605	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	3.0%	gil000232640	26299/9.57	27500/9.4	59
607	GJ21252 [*Drosophila virilis*]	GL-H-S01-0637-LF	5.0%	gil000021535	26816/9.39	26000/9.6	97
608	Novel					26000/9.6	
609	Hypothetical protein TTHERM_00420130 [*Tetrahymena thermophila*]	GlEp-N-N01-1117-LF	3.0%	gil000041476	25463/9.94	24500/9.6	56
624	Novel					48000/6.5	
625	Arginine kinase [*Penaeus monodon*]	GlEp-N-N01-0368-LF	15.0%	gil000037477	28024/8.72	47000/6.8	179
626	Novel					30700/6.45	
627	Unknown	ES-N-S02-0330-W	4.0%	gil000011466	26635/9.62	26700/5.9	48
628	28S ribosomal protein S16, mitochondrial [*Aedes aegypti*]	TT-N-S01-0017-W	3.0%	gil000232640	26299/9.57	14500/4.3	69

Table 3.2 (cont.) Characterized protein spots in testes of domesticated *P. monodon* broodstock (group B), (average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
629	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	13.0%	gi|000023037	27221/6.59	28500/5.2	158
630	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	8.0%	gi|000023037	27221/6.59	28000/5.45	77
639	Putative periplasmic protein involved in polysaccharide export [*Photobacterium profundum 3TCK*]		1.0%	gi|90414985	106842/5.30	26000/9.95	56

Table 3.3 Characterized protein spots in testes of domesticated *P. monodon* broodstock (group C), (average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
427	Hemocyanin [*Litopenaeus vannamei*]	HPA-N-N01-0089-LF	14.0%	gi|000150697	22842/5.84	36000/4.	152
448	Novel					17500/3.2	
449	Chd64 CG14996-PB [*Apis mellifera*]	AG-N-N01-0995-W	14.0%	gi|0000004382	29471/9.10	25000/8.6	237
450	Spectrin alpha chain, putative [*Pediculus humanus corporis*]	OV-N-S01-1451-W	3.0%	gi|000224334	28210/9.63	14800/4.7	50
451	Novel					26500/5.5	
452	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	13.0%	g|000023037	27221/6.59	27500/5.65	142
453	Glutathione peroxidase [*Scylla serrata*]	GL-H-S01-1009-LF	4.0%	gi|000023037	27221/6.59	28000/3.7	96
454	Novel					28000/5.5	
455	Novel					27500/4.45	
456	Novel					27800/5.1	
457	Novel					28200/5.4	
458	Novel					28200/4.8	
459	Novel					28200/4.55	
460	Novel					29500/4.8	
461	Novel					29800/4.65	
462	Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	TT-N-ST01-0056-W	14.0%	gi|000238206	26814/9.14	30200/4.95	137
463	Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	HC-N-N01-12735-LF	6.0%	gi|000093024	28421/8.80	3000/4.75	105
464	Novel					35000/4.3	
465	Farnesoic acid O-methyltransferase [*Penaeus monodon*]	IN-N-S01-1195-LF	5.0%	gi|000189097	28182/4.99	36000/4.35	65
466	Farnesoic acid O-methyltransferase [*Penaeus monodon*]	IN-N-S01-1195-LF	5.0%	gi|000189097	28182/4.99	37500/4.35	65
467	Farnesoic acid O-methyltransferase [*Penaeus monodon*]	IN-N-S01-1195-LF	7.0%	gi|000189097	28182/4.99	38000/4.4	91
468	Novel					40000/4.5	

Table 3.3 Characterized protein spots in testes of domesticated *P. monodon* broodstock (group C), (average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
469	Recombination activating protein 1 [*Sphenoeacus afer*]		1.0%	gil60460249	111507/8.63	39200/6.0	65
470	Novel					40000/5.8	
471	Novel					41000/5.8	
472	Hypothetical protein [*Monodelphis domestica*]	TT-N-ST01-0010-W	20.0%	gil000237941	13192/8.09	41000/5.95	76
473	Adenosine kinase 2 [*Culex quinquefasciatus*]	HPA-N-N01-0792-LF	3.0%	gil000153845	24514/9.72	44000/5.8	50
474	Novel					44500/5.9	
475	Novel					44900/5.9	
476	G protein gamma subunit [*Nasonia vitripennis*]	HC-V-S01-0001-LF	7.0%	gil000144727	16397/10.01	45000/6.0	53
477	Hemocyanin [*Litopenaeus vannamei*]	HPa-N-N03-0134-LF	11.0%	gil000158545	28324/5.99	44990/6.05	78
478	Unnamed protein product [*Homo sapiens*]		2.0%	gil28317	59720/5.17	44900/6.2	87
479	Novel					44000/6.3	
480	Nascent polypeptide-associated complex alpha [*Penaeus monodon*]	HPO-N-S01-0172-LF	6.0%	gil000064227	24050/5.05	37500/4.25	72
481	Novel					39000/4.1	
482	Novel					40200/4.2	
483	Calreticulin precursor [*Fenneropenaeus chinensis]*	HC-N-N01-12532-LF	13.0%	gil000091748	24947/4.86	42000/4.2	102
484	Novel		4.0%	gil18389889	46542/4.39	51000/4.2	77
487	C-type lectin protein [*Fenneropenaeus chinensis*]		5.0%	gil62126070	32559/4.51	47000/4.3	79
488	Novel					45000/4.5	
489	Protein disulfide isomerase [*Litopenaeus vannamei*]	OV-N-N01-0993-W	21.0%	gil000211587	25911/4.66	44000/4.65	183
490	Protein disulfide isomerase [*Litopenaeus vannamei*]	OV-N-S01-1324-W	12.0%	gil000223598	26256/5.42	52500/5.2	115
491	Novel					53000/5.25	

Table 3.3 Characterized protein spots in testes of domesticated *P. monodon* broodstock (group C), (average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
492	Novel					61000/5.85	
493	Novel					60000/5.85	
494	Novel					51000/6.2	
495	Unknown	LP-V-S01-0434-LF	8.0%	gil000200121	24880/9.58	52000/6.3	75
496	Unnamed protein product [*Tetraodon nigroviridis*]	HC-N-N01-4818-LF	3.0%	gil000113329	26601/9.10	47000/6.4	59
497	Novel					45500/6.35	
500	Novel					55000/6.95	
501	Novel					25100/4.8	
502	Novel					26000/4.65	
503	Novel					26000/3.8	
504	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	15.0%	gil000070688	23660/5.49	27500/5.05	148
505	Novel					26000/5.15	
506	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gil000070688	23660/5.49	27500/4.85	52
507	Novel					27500/4.7	
508	Novel					28500/4.8	
509	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	9.0%	gil000070688	23660/5.49	28300/4.6	74
510	Novel					28000/4.4	
511	Novel					29700/4.0	
512	14-3-3-like protein [*Penaeus monodon*]	AG-N-N01-0324-W	9.0%	gil0000001429	27959/5.06	31500/4.2	172
513	Novel						
514	Novel					36500/4.5	
515	Novel					37000/4.4	
516	Novel					36500/4.2	
517	Novel					37000/4.2	
518	Novel					37900/4.0	
519	Novel					37900/4.1	
520	Novel					40100/4.4	
521	Novel					40000/4.5	

Table 3.3 Characterized protein spots in testes of domesticated *P. monodon* broodstock (group C), (average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
522	Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]		7.0%	gil123477668	20547/9.73	40000/4.6	64
523	Novel					41000/4.15	
524	GI14833 [*Drosophila mojavensis*]	GlEp-N-N01-1931-LF	8.0%	gil000046263	29036/8.74	41000/4.5	63
525	Novel					41000/4.4	
526	Novel					48500/4.15	
527	Novel					49000/4.1	
528	Novel					48000/4.2	
529	Novel					37500/5.05	
530	Adenosine kinase 2 [*Culex quinquefasciatus*]	HPA-N-N01-0792-LF	3.0%	gil000153845	24514/9.72	41000/5.4	51
531	Novel					43000/5.45	
532	Novel					49000/5.45	
533	Novel					51000/5.45	
534	Novel					52000/5.5	
535	Novel					51000/5.9	
536	Novel					50000/6.05	
537	Novel					43000/6.5	
538	Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]		7.0%	gil123477668	20547/9.73	38500/8.6	60
539	Novel					36000/9.2	
540	Novel					15600/8.5	
541	Spectrin alpha chain, putative [*Pediculus humanus corporis*]	OV-N-S01-1451-W	3.0%	gil000224334	28210/9.63	14900/8.5	48
542	Novel					18000/9.5	
610	Ribosomal protein S6 [*Chaoborus sp. AF-2006]*	AG-N-N01-0276-W	3.0%	gil0000001205	27162/12.63	60000/4.5	47
611	Novel					60000/3.2	
612	Unknown	OV-N-N01-0669-W	7.0%	gil000209690	28769/8.70	60000/5.55	57

Table 3.3 Characterized protein spots in testes of domesticated *P. monodon* broodstock (group C), (average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02, *N* = 3)

Spot No.	Protein name	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
613	Protein-disulfide isomerase [*Scylla paramamosain*]	HC-N-N01-2411-LF	14.0%	gil000100935	25626/8.54	57500/5.5	132
614	Novel					43500/6.35	
615	Unnamed protein product [*Homo sapiens*]		3.0%	gil28317	59720/5.17	42800/6.4	154
616	Novel					37000/9.0	
617	Novel					32000/4.15	
618	Peroxiredoxin [*Fenneropenaeus indicus*]	HC-H-S01-0335-LF	5.0%	gil000070688	23660/5.49	28200/4.6	56
619	small heat shock protein ArHsp21 [*Artemia franciscana*]	AG-N-N01-0551-W	20.0%	gil0000002414	28638/6.36	26000/4.9	234
620	Novel					19100/4.1	
621	Novel					19200/4.8	
622	Novel					18500/4.8	
623	Novel					18000/4.6	
631	Adenosylhomocysteinase A [*Xenopus laevis*]	OV-N-N01-0647-W	11.0%	gil000209564	24676/5.98	46000/5.75	131
632	Adenosylhomocysteinase A [*Xenopus laevis*]	OV-N-N01-0647-W	11.0%	gil000209564	24676/5.98	46000/6.0	143
633	Novel					14400/4.3	
636	Novel					27500/5.1	
637	Novel					42500/5.75	
638	Novel					43000/5.75	
649	Novel					62000/3.01	
650	Novel					63000/3.0	
651	Novel					66000/3.0	

Protein spots significantly matched hypothetical proteins, unnamed proteins and unknown proteins in the database are regarded hypothetical proteins, unnamed proteins and unknown proteins, respectively. Protein spots that did not significantly match any sequence in the database were regarded as novel proteins.

Relatively low numbers of unnamed proteins, hypothetical proteins and unknown proteins were observed (15 spots, 2.34%; 32 spots, 4.99% and 31 spots, 4.84% respectively) across overall spots examined. Within each sample groups, unnamed proteins were found at 2.03, 2.50 and 3.17%, hypothetical protein were found at 5.06, 5.83 and 3.97% and unknown proteins were found at 5.06, 6.67 and 3.38%, respectively.

Table 3.4 A summary of similarity search of characterized protein spots in testes of *P. monodon* broodstock identified by NanoLC-MS/MS

Similarity search	WB-A (%)	DB-B (%)	DB-C (%)	Total spots (%)
Known protein	146 (37.06)	28 (23.33)	34 (26.98)	208 (32.50)
Unnamed protein	8 (2.03)	3 (2.50)	4 (3.17)	15 (2.34)
Hypothetical protein	20 (5.08)	7 (5.83)	5 (3.97)	32 (5.00)
Unknown protein	20 (5.08)	8 (6.67)	3(3.38)	31 (4.84)
Novel protein	200 (50.76)	74 (61.67)	80 (63.49)	354 (55.31)
Total	**394 (100)**	**120 (100)**	**126 (100)**	**640 (100)**

Note: WB-A = domesticated broodstock with the average GSI = 1.08 ± 0.18%, sperm sac/testis = 0.22 ± 01 (N = 3); DB-B = domesticated broodstock with the average GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 01 (N = 3); DB-C = domesticated broodstock with the average GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02 (N = 3)

Several different protein families were characterized. Reproduction-related proteins, for example, farnesolic acid-O-methyltransferase (FAMeT), progesterone receptor-related protein p23, receptor activating protein kinase C (RACK), 14-3-3-like protein, NADP-dependent leukotriene B4 12-hydroxydehydrogenase (LTB4DH) were found. In addition, heat shock proteins (hsp60), hsp70 and hsp90 were also identified.

Stress-related proteins such as glutathione peroxidase, trios-phosphate isomerase, cytosolic manganese superoxide dismutase and protein disulfide isomerase were also identified and characterized.

The glutathione-*s*-transferase (GST) enzymes are the best known for their role in detoxification of various exogenous compounds. These enzymes catalyze the

nucleophilic attack of the thiol group of GSH, γ-glutamylcysteinylglycine, at an electrophilic site of the second substrate. This reaction most frequently results in the covalent linkage of GSH to the second substrate, yielding a GSH conjugate, which generally less toxic than the parent compound (Irzyk et al., 1993).

Superoxide dismutases (SODs) are metalloproteins that catalyse the dismutation of superoxide radicals to oxygen and hydrogen peroxide. The enzyme has been found in all aerobic organisms examined, where it plays a major role in the defense against toxic reduced oxygen species which are generated in many biological oxidations (Brouwer et al., 2003).

Trios-phosphate isomerase (TPI) which is an enzyme in the Embden-Meyerhof-Parnas pathway of glycolysis. TPI is responsible for the reversible isomerization of dihydroxyacetone-phosphate and glyceraldehydes-3-phosphate.

Proteasome are highly complex protease responsible for selective protein degradation in eukaryotic cells. The 26S proteasome consists of two regulatory 19S cap complexes and 20S proteasome, which acts as the proteolytic or module subunit. The 26S proteasome degrades ubiquitinated proteins in an ATP-dependent reaction, whereas 20S proteasome alone does not exhibit that activity. It is thought that the 19s cap complexes of the 26S proteasome, which consist of ATPase, are associated with various cellular activities and non-ATPase subunits are recognition, unfolding and transport of a substrate protein to the proteolytically active 20S core (Gueckel et al., 1998).

From 2-DE and nanoESI-LC-MS/MS, three protein spots significantly matched proteasome alpha 4 subunit and proteasome delta of *Nasonia vitripennis* and proteasome subunit alpha type of *Aedes aegypti* were identified.

Table 3.5 Summary of proteins found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
14-3-3-like protein [*Penaeus monodon*]	438	B	3	AG-N-N01-0324-W	9.0%	gil0000001429	27959/5.06	32000/4.4	155
	512	C		AG-N-N01-0324-W	9.0%	gil0000001429	27959/5.06	31500/4.2	172
	599	B		AG-N-N01-0324-W	4.0%	gil0000001429	27959/5.06	32000/4.7	63
Arginine kinase [*Penaeus monodon*]	39	A	5	GlEp-N-N01-0368-LF	11.0%	gil000037477	28024/8.72	27000/4.1	148
	167	A		AG-N-N01-1003-W	7.0%	gil0000004411	28557/7.83	28557/7.83	68
	289	A		GlEp-N-N01-0368-LF	11.0%	gil000037477	28024/8.72	41000/6.1	152
	378	B		GlEp-N-N01-0368-LF	11.0%	gil000037477	28024/8.72	41000/6.25	93
	625	B		GlEp-N-N01-0368-LF	15.0%	gil000037477	28024/8.72	47000/6.8	179
Cyclophilin A [*Penaeus monodon*]	254	A	4	BT-N-S01-0099-W	7.0%	gil0000006266	20692/8.70	14000/6.1	80
	114	A		BT-N-S01-0099-W	12.0%	gil0000006266	20692/8.70	17500/9.1	142
	261	A		BT-N-S01-0099-W	26.0%	gil0000006266	20692/8.70	19000/8.7	148
	390	B		BT-N-S01-0099-W	31.0%	gil0000006266	20692/8.70	19000/9.0	211
Receptor for activated protein kinase c1 [*Penaeus monodon*]	101	A	1	AG-N-N01-0283-W	4.0%	gil0000001237	27631/6.25	37000/8.7	63
Farnesoic acid O-methyltransferase [*Penaeus monodon*]	465	C	3	IN-N-S01-1195-LF	5.0%	gil000189097	28182/4.99	36000/4.35	65
	466	C		IN-N-S01-1195-LF	5.0%	gil000189097	28182/4.99	37500/4.35	65
	467	C		IN-N-S01-1195-LF	7.0%	gil000189097	28182/4.99	38000/4.4	91
Expressed protein [*Arabidopsis thaliana*]	174	A	1	LP-Y-S01-0572-LF	6.0%	gil000203797	20943/9.59	29000/5.7	46
ATP binding / kinase/ protein serine/threonine kinase [*Arabidopsis thaliana*]	238	A	1		1.0%	gil15226197	79284/5.75	48000/4.95	60
Hemocyanin [*Litopenaeus vannamei*]	10	A	7	HPa-N-N03-0541-LF	28.0%	gil000160592	21257/5.34	80000/5.8	321
	11	A		HPa-N-N04-0536-LF	21.0%	gil000172285	27413/4.81	75000//5.8	200
	205	A		HPa-N-N03-0671-LF	5.0%	gil000161293	22080/5.98	80000/5.35	54
	427	B		HPA-N-N01-0089-LF	14.0%	gil000150697	22842/5.84	36000/4.8	152
	432	B		HPA-N-N01-0022-LF	10.0%	gil000150392	10473/6.45	28500/5.6	60
	435	B		HPa-N-N03-0541-LF	16.0%	gil000160592	21257/5.34	31000/5.8	150
	477	C		HPa-N-N03-0134-LF	11.0%	gil000158545	28324/5.99	44990/6.05	78

Table 3.5 (cont.) Summary of proteins found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Peroxiredoxin [*Fenneropenaeus indicus*]	63	A		OV-N-S01-0114-W	4.0%	gi|000216973	26185/5.81	29000/5.9	49
peroxiredoxin [*Penaeus monodon*]	76	A		HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	265005.2	52
	110	A		HC-H-S01-0335-LF	7.0%	gi|000070688	23660/5.49	27500/4.7	70
	198	A		HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	27000/5.0	57
	270	A		HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	28200/4.6	52
	410	A		HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	27300/5.2	57
	414	A		HC-H-S01-0335-LF	21.0%	gi|000070688	23660/5.49	27200/5.05	202
	419	B		HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	27500/5.3	58
	591	B		HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	27500/5.2	61
	504	C		HC-H-S01-0335-LF	15.0%	gi|000070688	23660/5.49	27500/5.05	148
	506	C		HC-H-S01-0335-LF	5.0%	gi|000070688	23660/5.49	27500/4.85	52
	509	C		HC-H-S01-0335-LF	9.0%	gi|000070688	23660/5.49	28300/4.6	74
40S ribosomal protein S2	242	A	1	OV-N-ST02-0027-LF	10.0%	gi|000231553	109147.16	49000/8.0	44
40S ribosomal protein [*Perinereis aibuhitensis*]	396	A	1	AG-N-N01-0667-W	11.0%	gi|0000002947	21176/8.41	17500/5.3	119
Ribosomal protein S6 *[Chaoborus sp. AF-2006*]	610	C	3	AG-N-N01-0276-W	3.0%	gi|0000001205	27162/12.63	60000/4.5	47
Putative ribosomal protein L32 [*Maconellicoccus hirsutus*]	36	A	1	HPA-N-N01-0643-LF	19.0%	gi|000153173	16322/11.72	49000/6.75	49
Ribosomal protein P2 [*Strongylocentrotus purpuratus*]	255	A	1	ES-N-S03-1095-W	48.0%	gi|000017899	14865/5.97	18500/3.7	388
28S ribosomal protein S16,	246	A	8	TT-N-S01-0017-W	3.0%	gi|000232640	26299/9.57	14400/4.1	65
mitochondrial [*Aedes aegypti*]	247	A		TT-N-S01-0017-W	3.0%	gi|000232640	26299/9.57	15500/4.1	59
	248	A		TT-N-S01-0017-W	3.0%	gi|000232640	26299/9.57	144000/4..4	68
	249	A		TT-N-S01-0017-W	3.0%	gi|000232640	26299/9.57	14400/4.7	56
	250	A		TT-N-S01-0017-W	3.0%	gi|000232640	26299/9.57	14400.5.2	73
	628	B		TT-N-S01-0017-W	3.0%	gi|000232640	26299/9.57	14500/4.3	69
	604	B		TT-N-S01-0017-W	8.0%	gi|000232640	26299/9.57	14500/5.0	120
	605	B		TT-N-S01-0017-W	3.0%	gi|000232640	26299/9.57	27500/9.4	59

Table 3.5 (cont.) Summary of proteins found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	Group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Small heat shock protein ArHsp21 [*Artemia franciscana*]	619	C	1	AG-N-N01-0551-W	20.0%	gil0000002414	28638/6.36	26000/4.9	234
70 kD heat shock protein [*Mirocaris fortunata*]	303	A	1	AG-N-N01-0802-W	5.0%	gil0000003572	26393/5.74	64000/4.9	63
Heat shock protein 60 [*Litopenaeus vannamei*]	206	A	2	OV-N-N01-0358-W	4.0%	gil000207891	26264/9.34	60000/5.0	77
	385	B		TT-N-S01-0846-W	8.0%	gil000236557	26532/5.11	57000/5.3	132
Hsp-90 [*Chiromantes haematocheir*]	7	A	1	HC-N-N01-12368-LF	4.0%	gil000090837	27112/9.07	87000/5.00	57
Fructose 1,6-bisphosphate aldolase [Artemia franciscana]	326	A	1	AG-N-N01-0522-W	6.0%	gil0000002294	27166/8.61		54
Heat shock protein gp96	3	A	1	OV-N-N01-0978-W	10.0%	gil000211496	27178/4.74	100000/4.6	146
Actin 2 [*Penaeus monodon*]	400	A	1	AG-N-N01-0779-W	3.0%	gil0000003476	26914/4.85	44500/4.7	49
Adenosine kinase 2 [*Culex quinquefasciatus*]	118	A	4	HPA-N-N01-0792-LF	3.0%	gil000153845	24514/9.72	40500/5.5	54
	445	B		HPA-N-N01-0792-LF	3.0%	gil000153845	24514/9.72	43000/5.9	49
	473	C		HPA-N-N01-0792-LF	3.0%	gil000153845	24514/9.72	44000/5.8	50
	530	C		HPA-N-N01-0792-LF	3.0%	gil000153845	24514/9.72	41000/5.4	51
Adenosylhomocysteinase A [*Xenopus laevis*]	210	A	3	OV-N-N01-0647-W	6.0%	gil000209564	24676/5.98	45000/5.7	61
	632	C		OV-N-N01-0647-W	11.0%	gil000209564	24676/5.98	46000/6	143
	631	C		OV-N-N01-0647-W	11.0%	gil000209564	24676/5.98	46000/5.75	131
Adenosylhomocysteinase [*Strongylocentrotus purpuratus*]	43	A	3	HPa-N-N03-1440-LF	4.0%	gil000165447	26692/5.33	45000/6.1	67
	44	A		HPa-N-N03-1440-LF	4.0%	gil000165447	26692/5.33	45000/5.9	61
	213	A		HPa-N-N03-1440-LF	11.0%	gil000165447	26692/5.33	45000/5.9	137
Alcohol dehydrogenase [*Bombyx mori*]	407	A	1	GL-N-STC02-0149-LF	7.0%	gil000030547	17936/5.73	44000/6.35	48
Aldolase [*Branchiostoma belcheri*]	441	B	1		3.0%	gil2244609	38811/8.11	43000/8.3	68
ATP synthase F0 subunit 6 *[Penaeus monodon*]	277	A	1	HPO-N-S01-0024-LF	5.0%	gil000063499	26914/9.44	27500/54.8	48

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Calreticulin precursor [*Fenneropenaeus chinensis*]	294	A	3	IN-N-S01-0553-LF	15.0%	gil000185623	21676/5.55	63000/3.7	144
	295	A		GlEp-N-S01-1589-LF	14.0%	gil000060373	16288/5.58	60000/3.8	94
	483	C		HC-N-N01-12532-LF	13.0%	gil000091748	24947/4.86	42000/4.2	102
Chaperonin containing TCP1, subunit 6A (zeta 1), isoform CRA b [Homo sapiens]	127	A	1	HC-W-S01-0248-LF	11.0%	gil000148503	12383/9.36	59000/7.1	91
Glycoprotein X precursor	8	A	1	HPa-N-N03-1190-LF	8.0%	gil000164062	24859/10.04		49
C-type lectin protein [*Fenneropenaeus chinensis*]	487	C	1		5.0%	gil62126070	32559/4.51	47000/4.3	79
Cytochrome b [*Litopenaeus stylirostris*]	220	A	1	HC-N-N01-3594-LF	6.0%	gil000106676	29347/9.26	14.700/5.3	46
Cytosolic malate dehydrogenase thermolabile form [*Sphyraena idiastes*]	283	A	1		5.0%	gil14583131	36463/6.60	40000/5.8	60
Cytosolic manganese superoxide dismutase [*Penaeus monodon*]	56	A	5	GlEp-N-S01-1341-LF	6.0%	gil000059095	25091/5.57	33000/5.7	83
	57	A		GlEp-N-S01-1341-LF	6.0%	gil000059095	25091/5.57	33500/5.5	86
	284	A		GlEp-N-S01-1341-LF	8.0%	gil000059095	25091/5.57	144000/4..4	97
	285	A		GlEp-N-S01-1341-LF	14.0%	gil000059095	25091/5.57	34000/4.9	187
	288	A		GlEp-N-S01-1341-LF	20.0%	gil000059095	25091/5.57	35000/5.1	244
Electron-transfer-flavoprotein, alpha polypeptide [*Danio rerio*]	286	A	1	HC-N-N01-0997-LF	4.0%	gil000079053	27958/8.41	34500/4.9	74
ENSANGP00000020121 [*Anopheles gambiae str. PEST*]	316	A	1	HC-H-S01-0530-LF	4.0%	gil000071804	27016/9.02	39300/5.4	59
Envelope glycoprotein [*Human immunodeficiency virus 1*]	578	B	1		3.0%	gil35396864	48265/9.33	39000/9.2	69
Epidermal cytokeratin 2 [*Homo sapiens*]	15	A	2		1.0%	gil181402	66111/ 8.07	63000/4.55	74
	25	A			1.0%	gil181402	66111/ 8.07	45000/5.4	86
Eukaryotic initiation factor 4A [*Callinectes sapidus]*	397	A	2	AG-N-N01-0171-W	5.0%	gil000000711	26870/4.83	49000/4.85	55
	398	A		AG-N-N01-0171-W	5.0%	gil000000711	26870/ 4.83	46000/4.95	58
F1-ATP synthase beta subunit [*Litopenaeus vannamei*]	19	A	1	HC-N-N01-13801-LF	9.0%	gil000098085	25085/5.62	51000/4.65	85

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
GDP dissociation inhibitor	402	A	1		4.0%	gil157492	51144/7.57	48000/5.0	84
GI14833 [*Drosophila mojavensis*]	524	C	1	GlEp-N-N01-1931-LF	8.0%	gil000046263	29036/8.74	41000/4.5	63
GJ21252 [*Drosophila virilis]*	607	B	1	GL-H-S01-0637-LF	5.0%	gil000021535	26816/9.39	26000/9.6	97
GJ21900 [*Drosophila virilis*]	416	B	1	HC-H-S01-0582-LF	26.0%	gil000072104	27806/7.25	20000/5.65	298
GK22671 [*Drosophila willistoni*]	442	B	2	TT-N-S01-0178-W	14.0%	gil000233415	17146/7.79	44000/8.55	154
	640	C		TT-N-S01-0178-W	7.0%	gil000233415	17146/7.79	45500/8.6	77
Glucose-regulated protein 78 [*Fenneropenaeus chinensis*]	12	A	1	OV-N-N01-0527-W	11.0%	gil000208861	26430/5.27	76000/5.0	139
Glutathione peroxidase [Scylla serrata]	68	A		GL-H-S01-1009-LF	4.0%	gil000023037	27221/6.59	26500/6.0	101
	72	A		GL-H-S01-1009-LF	4.0%	gil000023037	27221/6.59	26000/5.8	84
	173	A		GL-H-S01-1009-LF	4.0%	gil000023037	27221/6.59	26500/5.9	78
	279	A		GL-H-S01-1009-LF	4.0%	gil000023037	27221/6.59	28000/5.3	93
	280	A		GL-H-S01-1009-LF	9.0%	gil000023037	27221/6.59	28500/5.0	134
	630	B		GL-H-S01-1009-LF	8.0%	gil000023037	27221/6.59	28000/5.45	77
	452	C		GL-H-S01-1009-LF	13.0%	gil000023037	27221/6.59	27500/5.65	142
	453	C		GL-H-S01-1009-LF	4.0%	gil000023037	27221/6.59	28000/3.7	96
	642	C		GL-H-S01-1009-LF	12.0%	gil000023037	27221/6.59	44500/8.05	157
	629	C		GL-H-S01-1009-LF	13.0%	gil000023037	27221/6.59		158
Glutathione S-transferase Mu 3 [*Anoplopoma fimbria*]	64	A	1	AG-N-N01-0855-W	3.0%	gil0000003782	29371/6.12	28000/5.9	57
Glyceraldehyde 3-phosphate dehydrogenase [*Cambarus hamulatus*]	162	A	3	GL-H-S01-0820-LF	12.0%	gil000022387	25544/6.38	39000/7.2	124
	66	A		GL-H-S01-0663-LF	6.0%	gil000021673	25737/8.31	38000/7.4	48
	324	A		GL-H-S01-0663-LF	16.0%	gil000021673	25737/8.31	38000/6.9	170
Glycoprotein X precursor	8	A	1	HPa-N-N03-1190-LF	8.0%	gil000164062	24859/10.04	90000/5.00	49
Laminin receptor [*Litopenaeus vannamei*]	370	B	1	HC-W-S01-0862-LF	12.0%	gil000150266	19503/9.04	44000/4.8	121
Malate dehydrogenase (EC 1.1.1.37), mitochondrial - pig	409	A	1		7.0%	gil65932	33518/8.55	36500/7.3	95
Methylmalonate-semialdehyde dehydrogenase [*Aedes aegypti*]	311	A	1	GL-H-S01-1029-LF	7.0%	gil000023126	26676/6.51	53000/5.8	65

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
NADP-dependent leukotriene B4 12-hydroxydehydrogenase [*Gallus gallus*]	309	A	1	OV-N-N01-0347-W	4.0%	gil000207823	26428/7.11	42000/4.9	72
Nascent polypeptide-associated complex alpha [*Penaeus monodon*]	480	C	1	HPO-N-S01-0172-LF	6.0%	gil000064227	24050/5.05	37500/4.25	72
Nucleoplasmin isoform 1-like protein [*Maconellicoccus hirsutus*]	486	C	5	GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	24100/4.4	82
	499	C		GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	24100/4.6	57
	81	A		GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	22000.4.5	65
	265	A		GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	24500/4.6	67
	266	A		GL-H-S01-0626-LF	6.0%	gil000021469	21693/4.77	24500/4.5	67
Nucleotidase [*Pseudoalteromonas tunicata D2*]	209	A	1		4.0%	gil88859896	25095/4.79	48000/5.6	55
Pancreatic trypsin 1 [*Rattus norvegicus*]	375	B	1			gil6981420	26627	40000/5.4	63
PhoH-like protein [*Roseobacter phage SIO1*]	77	A	4		2.0%	gil19343479	43385/9.23	27000/5.2	70
	87	A			2.0%	gil19343479	43385/9.23	16000/4.7	66
	88	A			2.0%	gil19343479	43385/9.23	15500/4.0	66
	185	A			2.0%	gil19343479	43385/9.23	41000/5.8	58
Polarized growth protein [*Aspergillus fumigatus Af293*]	233	A	1		1.0%	gil7098924	109535/8.37	43000/7.25	60
Predicted protein [*Micromonas pusilla CCMP1545*]	21	A	3		3.0%	gil226458439	52600/5.76	53000/4.9	64
	237	A		ES-N-S03-0230-W	6.0%	gil000013568	16297/12.07	46000/5.1	56
	243	A		ES-N-S03-0230-W	6.0%	gil000013568	16297/12.07	54000/8.1	53
Proteasome alpha 4 subunit [*Nasonia vitripennis*]	59	A	1	HC-N-N01-13533-LF	3.0%	gil000096890	27577/8.12	31000/5.7	49
Proteasome delta [*Nasonia vitripennis*]	69	A	1	HC-N-N01-3568-LF	3.0%	gil000106520	26398/5.38	24000/6.1	72
Proteasome subunit alpha type [*Aedes aegypti*]	436	B	1	LP-N-N01-0262-LF	10.0%	gil000190425	20323/6.06	32000/5.6	121

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Protein disulfide isomerase [*Litopenaeus vannamei*]	14	A	7	OV-N-S01-1324-W	13.0%	gil000223598	26256/5.42	57000/4.5	269
	31	A		OV-N-S01-0764-W	13.0%	gil000220555	29075/5.60	58000/5.7	184
protein-disulfide isomerase [*Scylla paramamosain*]	207	A		OV-N-N01-0752-W	10.0%	gil000210181	24060/5.20	58000/5.5	132
	394	A		OV-N-N01-0752-W	24.0%	gil000210181	24060/5.20		258
	489	C		OV-N-N01-0993-W	21.0%	gil000211587	25911/4.66	44000/4.65	183
	490	C		OV-N-S01-1324-W	12.0%	gil000223598	26256/5.42	52500/5.2	115
	613	C		HC-N-N01-2411-LF	14.0%	gil000100935	25626/8.54	57500/5.5	132
Putative ABC transporter ATP-binding protein [*Streptomyces griseus subsp. griseus NBRC 13350*]	4	A	1		2.0%	gil182436389	73223/5.51	95000/4.7	58
Putative acidic p0 ribosomal protein [*Toxoptera citricida*]	58	A	3	GL-H-S01-0619-LF	4.0%	gil000021439	26882/9.07	36000/5.6	83
	287	A		GL-H-S01-0619-LF	9.0%	gil000021439	26882/9.07	37000/4.9	136
	374	B		GL-H-S01-0619-LF	8.0%	gil000021439	26882/9.07	38000/5.6	134
Putative oncoprotein nm23 [*Ictalurus punctatus*]	411	A	1		19.0%	gil000080101	23317/8.76	19500/7.5	239
Putative periplasmic protein involved in polysaccharide export [*Photobacterium profundum 3TCK*]	639	C	1		1.0%	gil90414985	106842/5.30	26000/9.95	56
Recombination activating protein 1 [*Sphenoeacus afer*]	469	C	1		1.0%	gil60460249	111507/8.63	39200/6.0	65
Rh type B glycoprotein [*Hylobates sp.*]	408	A	1		1.0%	gil17223572	49366/6.35	38500/5.7	63
Ribulose-1,5-bisphophate carboxylase/oxygenase small subunit [*Vitis pseudoreticulata*]	260	A	1		7.0%	gil86156014	20671/9.06	16000/8.4	97
Eukaryotic translation initiation factor 3 subunit E (Eukaryotic translation initiation factor 3 subunit 6) (eIF-3 p48) (eIF3e) (Viral integration site protein INT-6 homolog) [*Sus scrofa*]	297	A	1	AG-N-N01-0474-W	4.0%	gil0000002106	25099/8.95	57000/4.8	85
Chd64 CG14996-PB [*Apis mellifera*]	449	C	1	AG-N-N01-0995-W	14.0%	gil0000004382	29471/9.10	25000/8.6	237

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Sarcoplasmic calcium-binding protein [*Litopenaeus vannamei*]	264	A	2	AG-N-N01-0210-W	11.0%	gi|000000897	27761/5.55	25000/4.2	106
	603	B		AG-N-N01-0210-W	9.0%	gi|000000897	27761/5.55	25500/4.2	151
Furin-like protease 1, isoforms 1/1-X/2 precursor (Furin-1) (Kex2-like endoprote	310	A	1		3.0%	gi|91092736	88995/7.61	87000/4.75	135
G protein gamma subunit [*Nasonia vitripennis*]	476	C	1	HC-V-S01-0001-LF	7.0%	gi|000144727	16397/10.01	45000/6.0	53
Histone protein Hist2h3c1 [*Monodelphis domestica*]	232	A	2	ES-N-S03-0309-W	5.0%	gi|000013917	18784/11.27	44000/7.45	61
	240	A		ES-N-S03-0309-W	5.0%	gi|000013917	18784/11.27	49000/7.1	56
Intracellular fatty acid binding protein [*Penaeus monodon*]	116	A	3	ES-N-S01-0117-W	5.0%	gi|0000009405	26559/8.92	15000/5.7	107
	219	A		LP-N-N01-0788-LF	4.0%	gi|000192873	21988/7.75	21988/7.75	52
	395	A		LP-N-N01-0788-LF	11.0%	gi|000192873	21988/7.75	21988/7.75	141
p23-like protein [*Apis mellifera*]	176	A	2	HC-H-S01-0086-LF	8.0%	gi|000069261	20782/12.00	18000/4.2	47
	178	A		HC-H-S01-0086-LF	8.0%	gi|000069261	20782/12.00	39000/4.7	47
Mediator complex subunit 7 CG31390-PA isoform 1 [*Apis mellifera*]	32	A	1	HC-N-S01-0215-LF	6.0%	gi|000142347	33043/9.30	43000/6.7	55
Voltage-dependent anion-selective channel isoform 1 [*Triboliumcastaneum*]	103	A	2	AG-N-N01-1147-W	6.0%	gi|0000005006	20977/9.06	32000/9.5	52
	321	A		HPa-N-N03-1685-LF	21.0%	gi|000166545	26037/8.99	31000/9.3	216
Y43E12A.2	192	A	1	ES-N-S03-0713-W	5.0%	gi|000015867	27367/9.70		60
Spectrin alpha chain, putative [*Pediculus humanus corporis*]	450	C	2	OV-N-S01-1451-W	3.0%	gi|000224334	28210/9.63	14800/4.7	50
	541	C		OV-N-S01-1451-W	3.0%	gi|000224334	28210/9.63	14900/8.5	48
Substrate-binding transmembrane protein [*Ralstonia solanacearum GMI1000*]	24	A	1		1.0%	gi|17544781	86235/8.45	45000/5.3	65
Triosephosphate isomerase [*Fenneropenaeus chinensis*]	348	B	2	HC-N-N01-12801-LF	6.0%	gi|000093418	27631/9.42	28000/6	77
	569	B		HC-N-N01-7864-LF	33.0%	gi|000130550	25907/6.58	28500/5.8	243
Wdtc1 protein [*Mus musculus*]	228	A	1		3.0%	gi|22028134	39988/5.55	36000/4.2	58
Zinc-containing alcohol dehydrogenase [*Dictyostelium discoideum AX4*]	89	A	2	BT-N-S01-0482-W	4.0%	gi|0000007966	18704/9.82	24500/3.9	55
	95	A		BT-N-S01-0482-W	4.0%	gi|0000007966	18704/9.82	31000/4.15	47
Type II keratin subunit protein	108	A	1			gi|386854	52928	29000/4.8	71
Protease, serine, 1 [*Mus musculus*]	214	A	1		8.0%	gi|16716569	26802/4.75		104

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Trypsin precursor	70	A	4			gil136429	25078	24000/5.5	79
	98	A				gil136429	25078	17000/4.5	62
	106	A				gil136429	25078	32000/4.5	60
	188	A				gil136429	25078	41500.5.4	76
Tumor necrosis factor superfamily, member 5-induced protein 1	165	A	1	HC-N-N01-3133-LF	12.0%	gil000104189	18123/9.89	90000/6.15	53
Chain A, Crystal Structure Of Monomeric	28	A	2	BT-N-S01-0101-W	12.0%	gil0000006279	16659/5.01	43000/5.45	75
Actin Bound To Cytochalasin D	222	A			6.0%	gil40889964	16412/6.07	46000/6.8	58
Chain A, Crystal Structure Of Putative Holliday Junction Resolvase									
Chain E, Leech-Derived Tryptase	34	A	10		13.0%	gil3318722	24142/8.26	44500/6.65	134
InhibitorTRYPSIN COMPLEX	40	A			13.0%	gil3318722	24142/ 8.26	40500/6.2	106
	41	A			13.0%	gil3318722	24142/ 8.26	39000/6.25	108
	47	A			13.0%	gil3318722	24142/8.26	42000/4.5	122
	49	A			13.0%	gil3318722	24142/8.26	40000/6.0	128
	60	A			22.0%	gil3318722	24142/8.26	31000/5.5	170
	61	A			13.0%	gil3318722	24153/8.26	30000/5.4	114
	73	A			13.0%	gil3318722	24142/8.26	25000/5.6	125
	86	A			4.0%	gil3318722	24142/8.26	16000/4.7	63
	104	A				gil3318722	24142/8.26	35000/4.3	135
RecName: Full=Trypsin; Flags: Precursor	23	A	11		8.0%	gil136429	25078/7.00	50000/5.1	74
	33	A			8.0%	gil136429	25078/7.00	44000/6.55	63
	45	A			8.0%	gil136429	25078/7.00	48000/5.7	85
	48	A			8.0%	gil136429	25078/7.00	39000/4.65	65
	50	A			8.0%	gil136429	25078/7.00	40000/5.9	62
	51	A			8.0%	gil136429	25078/7.00	37000/6.1	62
	52	A			8.0%	gil136429	25078/7.00	36000/6.2	67
	53	A			8.0%	gil136429	25078/7.00	35500/6.0	83
	75	A			8.0%	gil136429	25078/7.00	26000/5.3	81
	83	A			8.0%	gil136429	25078/7.00	19000/5.7	62
	85	A			17.0%	gil136429	25078/7.00	17500/4.8	109

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Hypothetical peptide transporter ATP-binding protein [*Sulfolobus tokodaii str. 7*]	635	B	33		2.0%	gil15922871	35701/9.59	27500/5.15	56
Hypothetical protein BRAFLDRAFT_119287 [*Branchiostoma floridae*]	315	A		AG-N-N01-0134-W	4.0%	gil000000541	27356/6.95	42000/6.4	58
Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	462	C		TT-N-ST01-0056-W	14.0%	gil000238206	26814/9.14	30200/4.95	137
Hypothetical protein - bloodfluke planorb (fragment)	27	A		ES-N-S03-0155-W	5.0%	gil000013262	20441/9.83	43000/5.3	44
Hypothetical protein [*Monodelphis domestica*]	472	C		TT-N-ST01-0010-W	20.0%	gil000237941	13192/8.09	41000/5.95	76
Hypothetical protein [Thermobia domestica]	20	A		HPa-N-N02-0055-LF	8.0%	gil000154718	25872/9.93	54000/5.0	66
Hypothetical protein BradDRAFT_3909 [*Bradyrhizobium sp. BTAi1*]	200	A			3.0%	gil78696479	20528/7.88	28000/4.8	60
Hypothetical protein BRAFLDRAFT_114917 [*Branchiostoma floridae*]	302	A		AG-N-N01-0407-W	5.0%	gil0000001778	26755/6.66	53000/5.5	101
Hypothetical protein BRAFLDRAFT_115608 [*Branchiostoma floridae*]	296	A		OV-N-S01-1780-W	23.0%	gil000225962	27909/8.29	44000/6.2	184
Hypothetical protein BRAFLDRAFT_119287 [*Branchiostoma floridae*]	379	B		AG-N-N01-0134-W	4.0%	gil000000541	273566.95	43500/6.5	58
Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	79	A		HC-N-N01-12735-LF	6.0%	gil000093024	28421/8.80	29000/4.8	103
Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	331	A		TT-N-ST01-0056-W	14.0%	gil000238206	26814/9.14	29800/4.6	183

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	356	B		HC-N-N01-12735-LF	6.0%	gil000093024	28421/8.80	300000/5.0	111
Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	463	C		HC-N-N01-12735-LF	6.0%	gil000093024	28421/8.80	3000/4.75	105
Hypothetical protein BRAFLDRAFT_280892 [*Branchiostoma floridae*]	597	B		HC-N-N01-12735-LF	6.0%	gil000093024	28421/8.80	30000/4.8	104
Hypothetical protein BRAFLDRAFT_79044 [*Branchiostoma floridae*]	54	A		AG-N-N01-0546-W	4.0%	gil0000002391	26985/9.35	34000/6.0	70
Hypothetical protein BRAFLDRAFT_79044 [*Branchiostoma floridae*]	291	A		AG-N-N01-0546-W	5.0%	gil0000002391	26985/9.35	41000/4.9	97
Hypothetical protein BRAFLDRAFT_79044 [*Branchiostoma floridae*]	293	A		HC-N-N01-1115-LF	6.0%	gil000085199	27890/7.56	31500/6.0	63
Hypothetical protein BRAFLDRAFT_86061 [*Branchiostoma floridae*]	55	A		GlEp-N-N01-1607-LF	8.0%	gil000044355	29312/9.37	32500/6.0	133
Hypothetical protein CBG09936 [*Caenorhabditis briggsae*]	391	A			1.0%	gil39590708	96606/6.16	97000/5.1	90
Hypothetical protein LOC553452 [*Danio rerio*]	317	A		HC-N-S01-0103-LF	3.0%	gil000141719	28487/8.94	39000/5.4	66
Hypothetical protein Nham_3970 [*Nitrobacter hamburgensis X14*]	313	A			5.0%	gil92119376	19666/9.92	55000/5.9	70
Hypothetical protein Nwi_0969 [*Nitrobacter winogradskyi Nb-255*]	203	A			5.0%	gil75675162	27280/5.28	90000/5.2	62
Hypothetical protein TcasGA2_TC001048 [*Tribolium castaneum*]	392	A		HT-N-S01-0023-LF	9.0%	gil000061736	16159/4.93	19500/4.35	51

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
Hypothetical protein TcasGA2_TC001230 [*Tribolium castaneum*]	80	A		AG-N-N01-0313-W	4.0%	gil0000001379	28458/10.50	27000/4.5	49
Hypothetical protein TcasGA2_TC006408 [*Tribolium castaneum*]	579	B		AG-N-N01-0719-W	9.0%	gil0000003182	11090/7.85	36000/9.6	49
Hypothetical protein TcasGA2_TC014998 [*Tribolium castaneum*]	440	B		HT-N-S01-0297-LF	14.0%	gil000062817	11120/6.08	440007.6	98
Hypothetical protein TM1040_2050 [*Silicibacter sp. TM1040*]	259	A			7.0%	gil99081890	12396/10.09	16000/7.9	59
Hypothetical protein TTHERM_00420130 [*Tetrahymena thermophila*]	609	B		GlEp-N-N01-1117-LF	3.0%	gil000041476	25463/9.94	24500/9.6	56
Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]	94	A			7.0%	gil123477668	20547/9.73	17500/7.8	58
Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]	99	A			7.0%	gil123477668	20547/9.73	45000/5.15	64
Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]	522	C			7.0%	gil123477668	20547/9.73	40000/4.6	64
Hypothetical protein TVAG_137670 [*Trichomonas vaginalis G3*]	538	C			7.0%	gil123477668	20547/9.73	38500/8.6	60
Unnamed protein product [*Homo sapiens*]	164	A	15		1.0%	gil28317	59720/5.17	36000/6.65	64
	177	A			1.0%	gil28317	59720/5.17	18000/7.4	56
	197	A			3.0%	gil28317	59720/5.17	26000/5.1	131
	275	A			2.0%	gil28317	59720/5.17	27500/5.45	86
	307	A			2.0%	gil28317	59720/5.17	56000/5.7	89
	413	A			1.0%	gil28317	59720/5.17	27300/5.1	67
	418	B			6.0%	gil28317	59720/5.17	28000/5.2	255
	431	B			5.0%	gil28317	59720/5.17	28000/5.5	218
	437	B			1.0%	gil28317	59720/5.17	32000/5.65	70
	478	C			2.0%	gil28317	59720/5.17	44900/6.2	87
	484	C			4.0%	gil18389889	46542/4.39	51000/4.2	77
	615	C			3.0%	gil28317	59720/5.17	42800/6.4	154
	299	A			3.0%	gil124424210	41032/4.75	55000/5.0	71

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Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
	301	A			3.0%	gil124424210	41032/4.75	55000/5.2	67
	496	C		HC-N-N01-4818-LF	3.0%	gil000113329	26601/9.10	47000/6.4	59
Unknown	1	A	31	HC-N-N01-2578-LF	3.0%	gil000101580	28829 /9.65	96000/5.5	62
	2	A		GlEp-N-N01-2148-LF	15.0%	gil000047553	28541/9.11	95000/5.9	57
	18	A		BT-N-S01-0466-W	6.0%	gil0000007918	21082/9.61	51000/4.55	51
	29	A		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	65000/5.5	57
	30	A		OV-N-N01-0669-W	7.0%	gil000209690	28769/8.70	60000/5.2	47
	38	A		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	63000/6.3	49
	67	A		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	36000/7.55	45
	74	A		OV-N-N01-0669-W	7.0%	gil000209690	28769/8.70	26500/5.5	53
	90	A		ES-N-S03-0696-W	4.0%	gil000015766	27191/8.05	27000/4.1	49
	202	A		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	90000/5.1	46
	215	A		HC-H-S01-0193-LF	5.0%	gil000069873	23555/10.35	47000/6.1	50
	221	A		ES-N-S03-0550-W	4.0%	gil000015050	23237/10.29	14400/4.1	51
	235	A		AG-N-N01-0248-W	21.0%	gil0000001059	6634/5.52	45000/5.2	62
	282	A		OV-N-N01-0669-W	7.0%	gil000209690	28769/8.70	32200/4.0	61
	300	A		HC-N-N01-8453-LF	19.0%	gil000133797	6571/11.00	55000/5.1	76
	304	A		TT-N-S01-0497-W	7.0%	gil000235039	23572/6.64	4400/4.95	112
	334	A		BT-N-S01-0251-W	6.0%	gil0000006915	19989/8.33	32000/8.6	50
	383	B		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	65000/6.1	46
	389	B		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	19000/8.8	48
	393	A		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	19500/4.7	52
	399	A		AG-N-N01-0248-W	21.0%	gil0000001059	6634/5.52	44500/5.01	85
	401	A		AG-N-N01-0248-W	21.0%	gil0000001059	6634/5.52	44000/5.35	79
	485	C		ES-N-S03-0946-W	5.0%	gil000017155	28759/9.50	23500/4.4	52
	495	C		LP-V-S01-0434-LF	8.0%	gil000200121	24880/9.58	52000/6.3	75
	566	B		HC-N-N01-14005-LF	5.0%	gil000099077	20089/11.23	38500/4.65	56
	570	B		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	36500/6.7	75
	576	B		HPO-N-S01-0350-LF	7.0%	gil000065224	29726/9.74	39000/9.2	48
	592	B		OV-N-N01-0669-W	7.0%	gil000209690	28769/8.70	265000/5.0	48

Table 3.5 (cont.) Summary of protein spots found in testes of both wild and domesticated *P. monodon* broodstock

Protein nane	Spot No.	group	Total spot	Clone no.	Coverage	Accession no.	Theoretical Mr (Da/pI)	Observe Mr (Da/pI)	Mascot Score
	596	B		OV-N-N01-0056-W	2.0%	gi|000206110	26565/8.98	28500/4.9	52
	612	C		OV-N-N01-0669-W	7.0%	gi|000209690	28769/8.70	60000/5.55	57
	627	B		ES-N-S02-0330-W	4.0%	gi|000011466	26635/9.62	26700/5.9	48

A= testes from wild booodstock of *P.monodon* (GSI = 1.08 ± 0.18%, sperm sac/testis = 0.26 ± 0.06)

B = testes from domesticated booodstock of *P.monodon* group B (GSI= 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 0.01)

C = testes from domesticated booodstock of *P.monodon* group C (GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02)

3.2 One-dimensional polyacrylamide gel electrophoresis (SDS-PAGE)

3.2.1 Protein profiles during testicular development of *P. monodon*

For one-dimensional gel electrophoresis, total proteins were extracted from 6 individuals of wild male broodstock of *P. monodon* and samples were divided to two group according to the electrophoresed protein patterns (wild A, average body weight 123.55 ± 9.36 g, GSI = 0.66 ± 0.18%, and wild B, average body weight 120.67 ± 11.09 g, GSI = 0.68 ± 0.09%, N = 3 for each group). Three individuals each of 14-month-old (DB-14) (N = 3, average body weight = 69.84 ± 2.76 g, GSI = 0.37 ± 0.03%, and 18-month- old (DB-18) (N = 3, average body weight = 82.18 ± 2.88 g, GSI = 0.37 ± 0.01%) were also included in the experiments.

3.2.2 SDS-PAGE

Ten micrograms of total proteins from testes of each shrimp were analyzed by 12.5% SDS-PAGE. The electrophoresed bands were visualized by silver staining (Figure 3.10). The gel bands were excised according to the molecular mass range compared to protein standard markers and the in-gel trypsin digestion was performed. The extracted peptides of each molecular mass range sample were injected into LC-MS/MS

3.2.3 Protein annotation and functional classification

The raw data from LC-MS/MS were analyzed using DeCyder MS Differential Analysis software. The analyzed MS/MS data from DeCyder MS were submitted to database search using the Mascot. The data was searched against the NCBI database of animal for protein identification until 0 score of match proteins.

The intensity of the protein spectrum from testes of wild broodstock group A was used to normalized that of other sample groups. Based on the fact that a few thousands of different proteins were identified for each molecular weight range, approximately 50 proteins that showed large differential (up-regulation and down-regulation) expression profiles among sample groups were annotated in this Thesis.

In total, 345 differentially expressed proteins were identified, 223 (64.64%) of these significantly matched known proteins in the database and 122 (36.36%) proteins did not matched any protein in the NCBI database and were considered as unknown proteins (Table 3.6). Interestingly, 1 (0.29%) proteins were found in only wild broodstock groups A), 18 (5.22%) were found in both groups A and B broodstock but not in domesticated broodsotck while 231 (66.96%) proteins were commonly found in all groups of samples.

Protein found only in wild broodstock group A of *P. monodon* was GK24443 and those found in both groups of wild broodstock were. p97/VCP-binding protein p135, lipoxygenase homology domains 1, dipeptidyl-peptidase and SEParase family member (sep-1).

Examples of proteins that the expression level seems to be decreased in domesticated broodstock were zinc finger protein 184, glutathione S-transferase alpha 1, seven transmembrane helix receptor , cortactin-binding protein 2, nuclear receptor subfamily 3, group C, member 2 and syntaxin 5.

Several proteins seem to be more abundantly expressed in domesticated broodstock than wild broodstock. They were, for example, kinesin like protein 67a, RUN domain containing 2A, GPBP-interacting protein 130b and brain cyclic nucleotide gated 1.

In addition, known proteins in this study were further categorized according to the biological functions of their homologues using the Gene Ontology Categorizer (GoCat software) and 223 differentially expressed proteins identified in testes of wild and domesticated broodstock of *P. monodon* were able to be categorized to 11 functional categories (Figures 3.22)

These included transport and binding proteins (57 proteins accounting for 16.52%; e.g. arginine kinase 2 , brain cyclic nucleotide gated 1, asparagine-rich antigen, deltex 2 and DNA methyltransferase), biosynthetic process (7 protein proteins accounting for 2.03% including. 5'-nucleotidase, dedicator of cytokinesis family protein , GI13543, guanylate cyclase, phosphoribosylformylglycinamidine synthase (FGAR amidotransferase) isoform CRA_a, midasin homolog (yeast) and

regulatory solute carrier protein, family 1, member 1), catabolic process (4 proteins accounting for 1.16% including Uba1a protein, WW and C2 domain containing 2, inositol polyphosphate-4-phosphatase, type II, 105kD and ubiquitin specific peptidase 38), cell division/DNA synthesis, repair and replication (41 proteins accounting for 11.88%, e.g. Zinc finger protein 184, zinc finger RNA binding protein, transcription factor 25, serine/threonine protein kinase and RUN domain containing 2A), chaperone (2 proteins accounting for 0.58% including DnaJ homolog subfamily B member 1 (Heat shock 40 kDa protein 1) and GL21472), defense and homeostasis (11 proteins accounting for 3.19%, e.g. collagen type IV CG4145-PA, isoform A isoform 1,

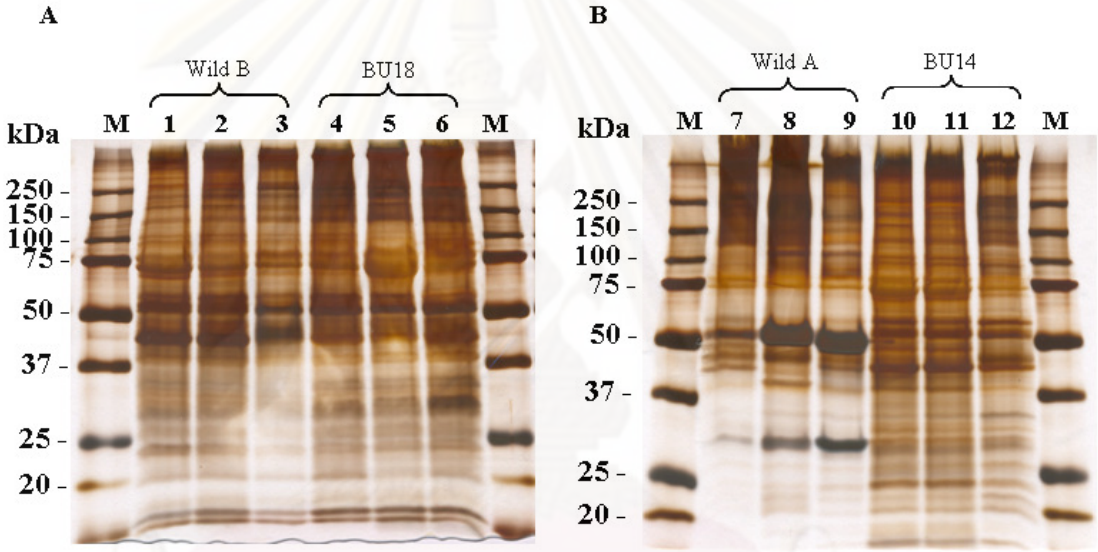

Figure 3.10 A 12.5% SDS-PAGE showing expression patterns of testes (panel A) of wild broodstock pattern B (lanes 1 - 3) and domesticated 18 months old (lanes 4 -6), (panel B), wild broodstock pattern A (lane 7 - 9) and domesticated 14 months old (lane 10 - 12). Lanes M is the protein marker.

peptidoglycan recognition protein-lc, immunity-related GTPase M9, ectonucleoside triphosphate and intersectin long isoform 1), metabolic process (22 proteins accounting for 6.38%, e.g. fatty-acid amide hydrolase, putative, ATP synthase subunit alpha, mitochondrial precursor, ATPase, H^+ transporting, lysosomal V0 subunit A2, glutathione S-transferase alpha 1 and phosphate transporter), oxidation-reduction (8 protein accounting for 2.32%, including 2,4-dichlorophenol hydroxylase, cytochrome P450, family 4, subfamily A, polypeptide 11, NADPH oxidase 4, NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa precursor isoform 1, oxidoreductase, short chain dehydrogenase/reductase family protein, CG4009 and GH16376), RNA processing (8 proteins accounting for 2.32%, including nuclear cap-binding protein subunit 1, nucleoporin 133, glutamyl-tRNA synthetase, glutamyl-tRNA cleavage and polyadenylation specificity factor 1, partial, initation factor 4B and sfrs8 protein), signal transduction (36 proteins accounting for 10.43%, e.g. Ran GTPase activating protein 1, vomeronasal type-1 receptor 1, F-box A protein family member (fbxa-218), Protein tyrosine phosphatase 99A CG2005-PB, isoform B and GTP-binding protein alpha subunit, gna) and structural protein (27 proteins accounting for 7.83%, e.g. giantin, calmodulin regulated, chromosome-associated kinesin KIF4A, dynamin and circadian clock protein PER3), respectively.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon*

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Transport and binding proteins							
gi|58392890	AGAP008942-PA [*Anopheles gambiae str. PEST*]	Transmembrane Transport	K.LGTPDAVPWLR.L	1	0.89	0.88	0.88
gi|91076670	AGAP012249-PA [*Tribolium castaneum*]	ATP binding	R.ILAGLGFTK.E	1	1.00*	1.05	1.05
gi|241896695	Arginine kinase 2 [*Ctenocephalides felis*]	ATP binding	K.GKFHPLTGMPK.D	1	0.86	0.83	0.87
gi|256080731	Asparagine-rich antigen [*Schistosoma mansoni*]	Tucleic acid binding	R.IAYATPALAK.A	1	1.11	1.10	1.11
gi|157822789	ATP-binding cassette, sub-family C (CFTR/MRP), member 10 [Rattus norvegicus]	Transmembrane Transport	R.QPQDTCR.L	1	0.79**	0.76*	0.84
gi|189521357	ATP-binding cassette, sub-family C (CFTR/MRP), member 12-like [*Danio rerio*]	Transport	K.TYMKDTISK.L	1	0.96**	1.06	1.03**
gi|62896547	B-cell receptor-associated protein 31 variant [*Homo sapiens*]	Tntracellular protein transport	K.QAEGASEAAKK.Y	1**	ND	0.91	0.86
gi|2708316	Brain cyclic nucleotide gated 1 [*Mus musculus*]	Ion transport	R.GGAAGK.E	1	1.00*	1.05	1.05
gi|241738304	Cell adhesion molecule, putative [*Ixodes scapularis*]	Calcium ion binding	M.HSVPETAPELK.S	1	1.04*	ND	0.96*
gi|180249	Ceruloplasmin [*Homo sapiens*]	Copper transport	K.VFNPR.R	1	1.14**	1.07	1.15
gi|66504484	CG10750-PA [*Apis mellifera*]	Protein binding	R.ARCELEK.T	1	ND	0.79*	ND
gi|156550321	CG3563-PA [*Nasonia vitripennis*]	Protein binding	K.LFSRPGWR.R	1	1.11	1.10	1.11
gi|198423371	Coatomer protein complex, subunit beta 2 [*Ciona intestinalis*]	ER-Golgi transport	K.ENLSSTNK.K	1	0.89*	1.07	0.97**

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Transport and binding proteins							
gi|109117530	Component of oligomeric golgi complex 1 isoform 3 [*Macaca mulatta*]	Protein transport	-.MAAAATSPALKR.L	1	1.02**	1.39	0.96**
gi|38322755	Cortactin-binding protein 2 [*Sus scrofa*]	Protein binding	K.TSGVGR.V	1	0.90	0.87**	0.88**
gi|118101683	CSMD2 protein [*Gallus gallus*]	Protein binding	R.VGTDLK.L	1	0.89**	0.92	0.96**
gi|118091399	Deltex 2 *[Gallus gallus*]	Protein binding	-.CLVLHPPPVSK.S	1	0.89	0.84	0.89
gi|66472506	DNA methyltransferase [*Danio rerio*]	Chromatin modification	R.AFGQHLQQSK.S	1	1.13**	1.04**	1.08
gi|198471057	GA16968 [*Drosophila pseudoobscura pseudoobscura*]	ATP binding	K.LDELNASEK.A	1	1.11	1.10	1.11
gi|211853279	GABAA receptor subunit rho 1 [Carassius carassius]	Ion transport	-.MTFDGRLVK.K	1	0.96	0.90	0.92
gi|198426437	GE16049 [*Ciona intestinalis*]	Ion transport	K.ASDDPK.A	1	0.89**	0.92	0.96**
gi|195502815	GE23982 [*Drosophila yakuba*]	Zinc ion binding	R.AELEEVVIAEAK.Q	1	0.91	0.88	0.87
gi|194761930	GF14089 [*Drosophila ananassae*]	ATP binding	R.ELTAISVTPGR.D	1	ND	1.15*	1.16*
gi|194763761	GF20958 [*Drosophila ananassae*]	ATP binding	K.AGGAAASGQDNGK.S	1	0.96**	1.06	1.03**
gi|194750741	GF23904 [*Drosophila ananassae*]	ATP binding	R.GLLDVVIVGALR.A	1	0.89	0.88	0.88
gi|195036100	GH18763 [*Drosophila grimshawi*]	RNA binding	K.DIMAALEK.A	1	ND	0.79*	ND

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Transport and binding proteins							
Gi|195120017	GI19982 [*Drosophila mojavensis*]	RNA binding	K.NLETSLLLLASK.E	1	0.91	0.88	0.87
Gi|195401905	GJ14766 [*Drosophila virilis*]	Transmembrane transport	R.VDEALATLRR.I	1	ND	1.15*	1.16*
Gi|195434947	GK14654 [*Drosophila willistoni*]	Lipid transport	K.LMNQLGKSK.L	1	1.11	1.10	1.11
Gi|195450070	GK22370 [*Drosophila willistoni*]	Nucleic acid binding	R.TSQGIK.T	1	0.89**	0.92	0.96**
Gi|195437490	GK24443 [*Drosophila willistoni*]	Nucleic acid binding	K.SSSSSSSSSSSSSGQRK.E	1	ND	ND	ND
Gi|195154869	GL17657 [*Drosophila persimilis*]	Nucleic acid binding	K.HAQPEPKV.-	1	0.79**	0.76*	0.84
Gi|195174305	GL27102 [*Drosophila persimilis*]	Protein binding	R.TQHRNAR.D	1	1.01*	0.90**	ND
Gi|31074381	Glutamate receptor subunit protein GluR3 [*Aplysia californica*]	Ion transport	K.VAFYYDSDEGLVR.L	1*	1.29*	ND	1.29*
Gi|73956382	Hook homolog 1 (h-hook1) (hHK1) [*Canis familiaris*]	Early endosome to late endosome transport	M.TSGALK.K	1	0.90	0.87**	0.88**
Gi|1881662	Kinesin like protein 67a [*Drosophila melanogaster*]	Centrosome separation	K.IKNINYR.Q	1	1.00*	1.05	1.05
Gi|21668096	Lactation elevated 1 [*Mus musculus*	ATP binding	R.NIPQFSLAK.R	1	1.11	1.10	1.11
Gi|94470463	Lipophorin receptor [*Galleria mellonella*]	Calcium ion binding	M.FVLVGCHRAAPK.F	1	0.92**	0.83**	0.97**

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Transport and binding proteins							
gi|73961693	Lipoxygenase homology domains 1 [*Canis familiaris*]	Protein binding	R.VSIGHGNVGVNR.G	1**	0.90**	ND	ND
gi|224047860	Lysosomal trafficking regulator [*Taeniopygia guttata*]	Protein transport	R.AAGDLMNTLK.S	1	1.30	1.31	1.25
gi|170593999	MKIAA0368 protein [Brugia malayi]	RNA binding	K.VLPATILIKLK.L	1**	0.90**	ND	ND
gi|154240734	Nuclear receptor subfamily 3, group C, member 2 [*Danio rerio*]	Cellular sodium ion homeostasis	K.TSGSPK.M	1	0.90	0.87**	0.88**
gi|198434236	P97/VCP-binding protein p135 [*Ciona intestinalis*]	Protein hexamerization	K.VYSFPLNKLK.C	1**	0.90**	ND	ND
gi|3057042	P-glycoprotein [*Haemonchus contortus*]	Transport	K.GVSLQVSAGQK.I	1	1.06	1.19	1.02
gi|49035804	Pol protein [*Oikopleura dioica*]	DNA integration	K.HIFSK.I	1	1.14**	1.07	1.15
gi|57169139	Putative pheromone receptor CPpr2 [*Cyprinidae sp*. EA-2004]	Transmembrane transport	K.SFHVLGGSLGFAMR.K	1**	0.79**	0.85**	ND
gi|114008	RecName: Full=Apolipoprotein A-IV; Short=Apo-AIV; Short=ApoA-IV; AltName: Full=Apolipoprotein A4; Flags: Precursor	Lipid transport	K.FNMALVQQMEK.F	1	0.92**	0.83**	0.97**
gi|54299675	Recombination activating protein 1 [*Trogon comptus*]	DNA recombination	K.KTPPDHAHPINK.D	1	0.92**	0.83**	0.97**
gi|118082971	Retinoblastoma binding protein 2 [*Gallus gallus*]	Histone H3-K4 demethylation	R.SEAFGMQMRQR.K	1	1.13	1.01**	1.19**

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Transport and binding proteins							
gi|170581535	SAND domain containing protein *[Brugia malayi*]	DNA binding	R.RLALMGPSQR.D	1	ND	1.15*	1.16*
gi|20151927	SD09067p [*Drosophila melanogaster*]	Receptor binding	R.TSGGK.E	1	0.89*	1.07	0.97**
gi|74011495	Solute carrier family 15, member 4 [*Canis familiaris*]	Oligopeptide transport	K.IDHTDDFR.W	1	1.11	1.10	1.11
gi|194213165	Solute carrier family 44, member 2 *[Equus caballus*]	Transmembrane transport	K.GPAES.-	1	1.13	1.12	1.04
gi|168334114	Sugar (Glycoside-Pentoside-Hexuronide) transporter [*Epulopiscium sp*. 'N.t. morphotype B']	Sodium ion transport	M.STTTETR.V	1*	0.76**	0.70*	ND
gi|55636219	Syntaxin 5 isoform 5 [*Pan troglodytes*]	Intracellular protein transport	K.HIGKDLSNTFAK.L	1	0.91**	0.85	0.86
gi|240995056	Zeta-associated protein Zap-70, putative [*Ixodes scapularis*]	protein binding	R.LQDSGHLDGKFL.-	1	0.91**	0.85	0.86
gi|189527995	Zinc finger protein 595, partial [*Danio rerio*]	Nucleic acid binding	K.KTFSCTQCGK.S	1	0.94	0.91	0.92
Biosynthetic process							
gi|152032120	5'-nucleotidase [*Ixodes scapularis*]	Purine nucleotide biosynthetic process	R.GANCSEK.K	1	0.95	0.91	0.90
gi|158293450	Guanylate cyclase [*Anopheles gambiae str. PEST*]	cGMP biosynthetic process	K.NDSSGMFKDK.S	1	ND	1.15*	1.16*

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Biosynthetic process							
gi|170584512	Dedicator of cytokinesis family protein [*Brugia malayi*]	Endomembrane system	K.ILAAMITR.Y	1	0.79**	0.76*	0.84
gi|195115481	GI13543 [*Drosophila mojavensis*]	'de novo' IMP biosynthetic process	R.RAPEYK.A	1	0.94	0.89	0.84*
gi|198415806	Midasin homolog (yeast), partial [*Ciona intestinalis*]	regulation of protein complex assembly	K.NKTNYNAEDWIQK.S	1*	1.18**	1.12*	ND
gi|119610473	Phosphoribosylformylglycinamidine synthase (FGAR amidotransferase), isoform CRA_a [*Homo sapiens*]	'de novo' IMP biosynthetic process	K.EAPEPGMEVVK.V	1	1.05	1.02*	1.04*
gi|5730021	Regulatory solute carrier protein, family 1, member 1 [*Homo sapiens*]	Intestinal absorption	R.STQGLK.F	1	0.89**	0.92	0.96**
Catabolic process							
gi|28958137	Uba1a protein [*Xenopus laevis*]	Ubl conjugation pathway	R.VGTETEK.V	1	0.94	0.89	0.84*
gi|189514882	Ubiquitin specific peptidase 38 [*Danio rerio*]	Ubiquitin-dependent protein catabolic process	K.KVMEAAEK.E	1	ND	0.79*	ND
gi|156717344	WW and C2 domain containing 2 [*Xenopus (Silurana) tropicalis*]	Modification-dependent protein catabolic process	K.VMLRQVEK.Q	1	1.11	1.10	1.11
gi|73983934	Inositol polyphosphate-4-phosphatase, type II, 105kD [*Canis familiaris*]	Polyphosphate catabolic process	K.ENLPFLNAVLK.N	1	0.89	0.94	1.03
Cell division / DNA synthesis, repair and replication							
gi|91088531	Beta nu integrin subunit AgBnu [*Tribolium castaneum*]	Cell adhesion	R.GSMCSNAR.I	1	0.92	0.99	1.00
gi|6694635	Brca1 [*Pteropus rayneri*]	DNA repair	K.ILIFGEGR.G	1	0.79**	0.76*	0.84
gi|170591925	Bromodomain containing protein [*Brugia malayi*]	DNA repair	K.VSSHESMPTSPSSAK.L	1**	0.96**	0.96*	1.01*

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Cell division / DNA synthesis, repair and replication							
gi|268565027	C. briggsae CBR-TPXL-1 protein [*Caenorhabditis briggsae*]	Embryonic development ending in birth or egg hatching	R.APVSVSKMTPK.N	1	ND	1.15*	1.16*
gi|126343449	Calcium/calmodulin-dependent protein kinase kinase 1, alpha, [*Monodelphis domestica*]	Cell differentiation	R.ISSAPSLSTR.D	1	1.11	1.10	1.11
gi|91088063	DNA-directed RNA polymerase [*Tribolium castaneum]*	Transcription	R.ANFHDHYLK.Q	1	ND	1.15*	1.16*
gi|242005339	Conserved hypothetical protein [*Pediculus humanus corporis*]	Regulation of transcription, DNA-dependent	K.STTPFDK.K	1*	0.76**	0.70*	ND
gi|194674457	Dachsous 2 (Drosophila) [*Bos taurus*]	Cell morphogenesis involved in differentiation	R.DGGAAPEVATVR.L	1	1.13**	1.04*	1.08
gi|242017225	DNA polymerase epsilon, catalytic subunit A, putative [*Pediculus humanus corporis*]	DNA replication initiation	K.LMLDDGPYKR.S	1	1.04*	ND	0.96*
: gi|1297340	DNA polymerase gamma [*Mus musculus*]	DNA repair	R.VGSELK.A	1	0.89**	0.92	0.96**
gi|198422875	DNA replication licensing factor MCM6 (Mis5 homolog) [*Ciona intestinalis*]	DNA replication initiation	R.AEAVEMAQAGDR.C	1	0.89	0.84	0.89
gi|115916049	DNA-repair protein complementing XP-A cells homolog (*Xeroderma pigmentosum*)	Nucleotide-excision repair	R.DGSKSEAPMDR.V	1**	0.90**	ND	ND
gi|17536339	ECT2 (mammalian Rho GEF) homolog family member (ect-2) [*Caenorhabditis elegans*]	Cell morphogenesis	R.SDVAMMFGK.L	1**	0.98*	ND	ND

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Cell division / DNA synthesis, repair and replication							
gi|242007396	Endonuclease/reverse transcriptase, putative [*Pediculus humanus corporis*]	RNA-dependent DNA replication	R.VLPLGEEDR.V	1	0.91	0.93	0.93**
gi|156555229	Gag-Pol [*Nasonia vitripennis*]	DNA repair	K.LKTALAELGVK.H	1	ND	1.15*	1.16*
gi|194758854	GF14813 [*Drosophila ananassae*]	Cell adhesion	R.STEDTSR.K	1*	0.76**	0.70*	ND
gi|194865516	GG14977 *[Drosophila erecta*]	DNA repair	K.LENSRGITK.S	1	1.11	1.10	1.11
gi|195107565	GI23661 [*Drosophila mojavensis*]	Regulation of transcription	R.VCTPNDAISK.V	1	0.92	0.97**	0.94
gi|114638239	LRP16 protein isoform 2 [*Pan troglodytes*]	Transcription	R.AGGGAQ.-	1	1.13	1.12	1.04
gi|126273299	M-phase phosphoprotein 1, [*Monodelphis domestica*]	Cell cycle arrest	R.DLQQGISEK.E	1	1.11	1.10	1.11
gi|77736297	NK2 transcription factor related, locus 3 [*Bos taurus*]	Sequence-specific DNA binding	K.KPLEAAGDCK.A	1**	ND	0.91	0.86**
gi|47523410	Nuclear factor of activated T-cells, cytoplasmic 1 [*Sus scrofa*]	Regulation of transcription, DNA-dependent	K.QSAASCPVLGGKR.M	1	0.91**	0.85	0.86
gi|66516204	Protein disabled [*Apis mellifera*]	Differentiation	R.NIDMIYGESR.S	1*	1.15*	1.20	ND
gi|118360405	Protein kinase domain containing protein [*Tetrahymena thermophila*]	Cell cycle arrest	R.STEDSEK.D	1*	0.76**	0.70*	ND
gi|113215	RecName: Full=Actin, clone 205	DNA repair	K.EITALAPSTIK.I	1	ND	1.15*	1.16*
gi|5811587	TIP120-family protein TIP120B, short form [*Rattus norvegicus*]	Regulation of transcription	R.SGEVQNLAVK.C	1	1.03	0.98	1.03**
gi|113983	RecName: Full=DNA-(apurinic or apyrimidinic site) lyase; AltName: Full=Apurinic-apyrimidinic endonuclease 1; Short=AP endonuclease 1; AltName: Full=APEX nuclease; Short=APEN	DNA repair	K.TSPSGK.S	1	0.90	0.87**	0.88**

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon*

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Cell division / DNA synthesis, repair and replication							
gi|1351421	RecName: Full=Wee1-like protein kinase	Cell division	R.QTHFQPNGK.R	1**	0.91	0.94	0.89
gi|114665043	RUN domain containing 2A [*Pan troglodytes*]	Cell cycle	R.AGGVR.D	1**	1.02	1.02	1.12**
gi|17508711	SEParase family member (sep-1) [*Caenorhabditis elegans*]	Embryonic development ending in birth or egg hatching	K.SLTGIDKLR.Q	1**	0.98*	ND	ND
gi|256070818	Serine/threonine protein kinase [S*chistosoma mansoni*]	Cell cycle	K.LVIQKCEK.I	1	1.11	1.10	1.11
gi|73955104	Serine/threonine-protein kinase SIK3 [*Canis familiaris*]	Cell cycle	K.TLRVGAPPSMPR.A	1	ND	ND	1.10*
gi|114608855	Solute carrier family 22, member 16 isoform 4 [*Pan troglodytes*]	Cell differentiation	R.VSNSPTEVQK.H	1**	ND	0.91	0.86**
gi|149636716	Suppressor of hairy wing homolog 4 (Drosophila) [*Ornithorhynchus anatinus*]	Chromatin modification	K.KQNTWMASSTK.S	1	ND	ND	1.10*
gi|126314598	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2 [MONODELPHIS DOMESTICA]	Neurogenesis	R.VAVIQFSEDPR.V	1**	0.92	0.88**	ND
gi|256086193	Thyroid hormone receptor-associated protein [*Schistosoma mansoni*]	Regulation of transcription from RNA polymerase II promoter	R.HLTTSGGGAGNVSR.S	1**	1.09	1.14*	ND
gi|148679789	Transcription factor 25 (basic helix-loop-helix), isoform CRA_b [*Mus musculus*]	Transcription regulation	R.LSGPMSRR.A	1**	0.87*	ND	ND
gi|40891625	Vasa-like protein [*Crassostrea gigas*]	Differentiation	K.EGGFGGGGFGSK.N	1**	0.91	0.94	0.89
gi|38424	Zinc finger protein [*Homo sapiens*]	Transcription	R.GGKCSTR.C	1	0.95	0.91	0.90
gi|109041366	Zinc finger protein 184 (Kruppel-like) [*Macaca mulatta*]	Transcription	K.ITLVQHQR.V	1**	0.99	0.96	0.92
gi|126323186	Zinc finger RNA binding protein [*Monodelphis domestica*]	Transcription	R.HIMSK.H	1	1.14**	1.07	1.15

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Chaperone							
gil109123709	DnaJ homolog subfamily B member 1 (Heat shock 40 kDa protein 1) (Heat shock protein 40) (HSP40) (DnaJ protein homolog 1) (HDJ-1) [*Macaca mulatta*]	Chaperone mediated protein folding requiring cofactor	R.GAGGAK.G	1	1.13	1.12	1.04
gil195153230	GL21472 [*Drosophila persimilis*]	Protein folding	K.SLPTATVSK.S	1**	0.88**	ND	0.91*
Defense and homeostasis							
gil110764127	Collagen type IV CG4145-PA, isoform A isoform 1 [*Apis mellifera*]	Angiogenesis	K.GEQGLPGLPGHKGER.G	1**	0.96**	0.96*	1.01*
gil146455221	complement factor B [*Triakis scyllium*]	Complement activation	K.ENDSSNSIGRK.L	1	1.04*	ND	0.96*
gil157822881	Complement factor properdin [*Rattus norvegicus*]	Complement activation	R.GGQCSEK.A	1	0.95	0.91	0.90
gil61098350	Ectonucleoside triphosphate diphosphohydrolase 1 [*Gallus gallus*]	Blood coagulation	R.LENKDAAEK.V	1	1.11	1.10	1.11
gil109123352	GATA binding protein 2 [Macaca mulatta]	Phagocytosis	K.DTQTPISQK.D	1	1.11	1.10	1.11
gil226875108	Immunity-related GTPase M9 [*Microcebus murinus*]	autophagy	K.VEAMSIEK.A	1	ND	0.79*	ND
gil126325247	Intersectin long isoform 1 [*Monodelphis domestica*]	Negative regulation of neuron apoptosis	K.DSAEVPGASGK.A	1	1.11	1.10	1.11
gil170042997	Peptidoglycan recognition protein-lc [*Culex quinquefasciatus*]	Antibacterial humoral response	K.ASSGTSSTSDARAR.R	1	0.92**	0.83**	0.97**
gil3130161	Pheromone receptor [*Takifugu rubripes*]	Cell projection assembly	K.HLMSK.S	1	1.14	1.07	1.15
gil147902292	TTK protein kinase [*Xenopus laevis*]	Anatomical structure homeostasis	R.KPLLNMSAK.T	1	1.11	1.10	1.11
gil154816109	Prolactin receptor [*Bufo japonicus*]	T cell activation	R.EPQCQHMK.V	1**	0.91	0.94	0.89

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Metabolic process							
gi|260426913	4-alpha-glucanotransferase [*Citreicella sp. SE45*]	Carbohydrate metabolic process	R.MQALEDEFR.A	1*	0.82*	0.83*	ND
gi|4757810	ATP synthase subunit alpha, mitochondrial precursor [*Homo sapiens*]	ATP synthesis coupled proton transport	R.NALGSSFIAAR.N	1	0.90**	0.93	0.94
gi|45382621	ATPase, H^+ transporting, lysosomal V0 subunit A2 [*Gallus gallus*]	ATP synthesis coupled proton transport	R.VAVVEGLNVR.I	1	0.97	1.01*	0.95*
gi|109892238	Cytochrome oxidase subunit I [*Rotaria tardigrada*]	Aerobic respiration	R.NSGAS.-	1*	0.72**	0.71**	0.83**
gi|241630722	Fatty-acid amide hydrolase, putative [*Ixodes scapularis*]	Amidase activity	R.LPATACPVGLGRK.S	1	0.85	0.89	0.91
gi|195135421	GI16602 [Drosophila mojavensis]	Metabolic process	R.CYAQGKR.I	1	1.01*	0.90**	ND
gi|148236007	Glutathione S-transferase alpha 1 [*Xenopus laevis*]	Metabolic process	K.TVLNM.-	1	0.90	0.87**	0.88**
gi|122796	RecName: Full=Hemocyanin B chain	Metabolic process	R.HWFSLFNTR.Q	1	1.04*	ND	0.96*
gi|115672059	Uncharacterized protein C20orf152 homolog [Strongylocentrotus purpuratus]	Alternative splicing	R.VCTPR.D	1	1.14**	1.07	1.15
gi|90968578	ADAM metallopeptidase domain 12 [*Xenopus* (*Silurana*) *tropicalis*]	Proteolysis	K.DLDSSLEK.G	1	ND	0.79*	ND
gi|91788778	Adenylate cyclase [*Polaromonas sp. JS666*]	G-protein signaling, coupled to cAMP nucleotide second messenger	K.KTASP.-	1**	0.94*	0.85**	ND
gi|158286973	AGAP005270-PA [*Anopheles gambiae str. PEST*]	Phosphoprotein phosphatase activity	K.VMMVGSLESDIK.E	1	0.96	0.94	0.98
gi|47717150	Cadherin A1 [*Ostrinia nubilalis*]	Homophilic cell adhesion	R.GSAIGRLVVQEIR.D	1	0.88**	0.85**	1.01**

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Metabolic process							
gi|291228855	Cubilin-like [*Saccoglossus kowalevskii*]	Cholesterol metabolic process	R.RPAYTR.L	1	0.94	0.89	0.84*
gi|193636769	Dipeptidyl-peptidase [*Acyrthosiphon pisum*]	Proteolysis	K.VNGAYSDNKIK.N	1**	0.90**	ND	ND
gi|60302716	Euchromatic histone-lysine N-methyltransferase 1 [*Gallus gallus*]	Histone methylation	K.KATAANAEVK.V	1**	0.98*	ND	ND
gi|195132625	GI21537 [*Drosophila mojavensis*]	Hydrolase	R.ITALGTEIR.Q	1	1.02**	0.89	1.00
gi|60359846	mKIAA0218 protein [*Mus musculus*]	Endodeoxyribonuclease activity,	R.DGPSRSGEGR.S	1	1.11	1.10	1.11
gi|170032688	Phosphate transporter [*Culex quinquefasciatus*]	Glycerol metabolic process	R.GMQGLK.W	1	0.89**	0.92	0.96**
gi|38304370	phosphofructokinase [Ascaris suum]	Glycolysis	K.DLLAAGRITAEK.A	1	0.89	0.94	1.03
gi|193624662	Serine protease [*Acyrthosiphon pisum*]	Proteolysis	K.TSGAGIK.F	1	0.89**	0.92	0.96**
gi|170065983	Tryptase [*Culex quinquefasciatus*]	Proteolysis	K.TYVSTAKK.L	1	0.92	0.99	1.00
oxidation reduction							
gi|2599295	2,4-dichlorophenol hydroxylase [*Burkholderia cepacia*]	Oxidation reduction	K.RALSVH.-	1*	ND	1.32**	ND
gi|24647576	CG4009 [Drosophila melanogaster]	Oxidation reduction	R.KINIAQFQK.I	1**	ND	0.91	0.86**
gi|118150926	Cytochrome P450, family 4, subfamily A, polypeptide 11 [*Bos taurus*]	Oxidation reduction	M.SVSALSPSR.A	1**	0.88**	ND	0.91*
gi|195015925	GH16376 [*Drosophila grimshawi*]	Oxidation reduction	K.LITEKASTR.V	1	1.11	1.10	1.11
gi|195128867	GI11568 [*Drosophila mojavensis*]	Oxidation reduction	K.LITQKASTR.I	1	1.11	1.10	1.11

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Oxidation reduction							
gi|57110953	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa precursor isoform 1 [*Canis familiaris*]	Oxidation reduction	R.MSSGVTGDWK.V	1	0.96	0.90	0.92
gi|125505611	NADPH oxidase 4 [*Ovis aries*]	Oxidation reduction	R.GKTVG.-	1**	1.02**	0.98	ND
gi|170589826	Oxidoreductase, short chain dehydrogenase/reductase family protein [*Brugia malayi*]	Oxidation reduction	R.LGNQAASMSTGR.W	1	0.88	0.91	0.88**
RNA processing							
gi|149512998	Cleavage and polyadenylation specificity factor 1, partial [*Ornithorhynchus anatinus*]	mRNA processing	R.DSGADK.Q	1	ND	0.95**	0.88*
gi|861468	DNA-depenent RNA polymerase largest subunit homolog [*Invertebrate iridescent virus 6*]	RNA elongation from RNA polymerase II promoter	K.DVGMK.I	1	1.16	1.15	ND
gi|226226665	Glutamyl-tRNA synthetase [*Gemmatimonas aurantiaca T-27*]	Glutamyl-tRNA aminoacylation	R.STDESTR.A	1*	0.76**	0.70*	ND
gi|156098077	Glutamyl-tRNA(Gln) amidotransferase subunit A [*Plasmodium vivax SaI-1*]	Translation	R.STLMSEK.V	1*	0.76**	0.70*	ND
gi|288100	Initation factor 4B [*Homo sapiens*]	Regulation of translational initiation	R.GGGDR.Y	1	1.13	1.12	1.04
gi|226479212	Nuclear cap-binding protein subunit 1 [*Schistosoma japonicum*]	Gene silencing by RNA	R.IAETASQSRGR.R	1	0.93**	1.01*	0.93*
gi|23274108	Nucleoporin 133 [Mus musculus]	mRNA export from nucleus	R.GTPMSTR.L	1	0.95	0.91	0.90
gi|160773669	Sfrs8 protein [*Xenopus (Silurana) tropicalis*]	RNA processing	K.FTMYSGAKGK.K +	1	0.90**	0.93	0.94

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Signal transduction							
gi|115772558	Ankyrin 2,3/unc44, partial [*Strongylocentrotus purpuratus*]	Signal transduction	M.PLEFR.M	1**	1.21*	1.10*	ND
gi|170033969	CDK10/11 [*Culex quinquefasciatus*]	Protein amino acid phosphorylation	R.SASPRSDQEGGR.R	1	0.89	0.84	0.89
gi|189241815	CG31304-PA [*Tribolium castaneum*]	Signal transduction	K.LHSNMAGSGK.Q	1	1.11	1.10	1.11
gi|109103507	Docking protein 1 isoform 2 [*Macaca mulatta*]	Transmembrane receptor protein tyrosine kinase signaling pathway	K.SGASGS.-	1**	0.83**	0.84	0.77*
gi|17556064	F-box A protein family member (fbxa-218) [*Caenorhabditis elegans*]	Auxin mediated signaling pathway	R.RDETSR.G	1	0.94	0.89	0.84*
gi|148232323	FK506 binding protein 6, 36kDa [*Xenopus laevis*]	Protein folding	K.QREMCCR.M	1**	0.91	0.94	0.89
gi|195571915	GD20700 [*Drosophila simulans*]	Signal transduction	K.ESKSMDDLEATK.E	1	0.93	1.44**	0.85*
gi|195024413	GH21048 [*Drosophila grimshawi*]	Protein amino acid phosphorylation	K.IIECIEK.D	1	0.79**	0.76	0.84
gi|195155789	GL25765 [*Drosophila persimilis*]	Signal transduction	K.QMMEKYLPR.S	1	0.94	0.92	0.93*
gi|118088380	Gravin [*Gallus gallus*]	bleb assembly	K.SDGKPEPTHLK.Q	1**	0.90**	ND	ND
gi|170029880	GTP-binding protein alpha subunit, gna [*Culex quinquefasciatus*]	G-protein coupled receptor protein signaling pathway	R.HVDGGGGGAR.G	1	1.01*	0.90**	ND
gi|259907203	Methyl-accepting chemotaxis protein [*Erwinia pyrifoliae Ep1/96*]	Chemotaxis	R.GAEVVSYVMEK.M	1	0.94	0.92	0.93*
gi|157133330	Mitogen activated protein kinase kinase 2, mapkk2, mek2 [*Aedes aegypti*]	Protein amino acid phosphorylation	R.NASPN.-	1**	0.94*	0.85**	ND

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Signal transduction							
gi|31872094	NEPH1 protein [*Homo sapiens*]	Excretion	-.MGQGLK.A	1	0.89**	0.92	0.96**
gi|221116657	Pk92B, partial [*Hydra magnipapillata*]	Protein amino acid phosphorylation	R.GVSTFKK.D	1	0.95	0.91	0.90
gi|156349526	Predicted protein [*Nematostella vectensis*]	Protein amino acid phosphorylation	R.DLKVSNLLLTGK.G	1	0.91	0.88	0.87
gi|156383548	Predicted protein [*Nematostella vectensis*]	Protein amino acid phosphorylation	K.GMDSRIQSLGGEGVK.R	1**	0.96**	0.96*	1.01*
gi|156351143	Predicted protein [*Nematostella vectensis*]	Protein amino acid phosphorylation	R.NATKHIK.I	1	0.99**	0.95**	0.90*
gi|156405274	Predicted protein [*Nematostella vectensis*]	Protein amino acid phosphorylation	K.LLAQKADLEK.V	1	0.96	0.94*	0.91**
gi|156390910	Predicted protein [*Nematostella vectensis*]	Protein amino acid phosphorylation	K.QSSIFSSMGK.G	1**	ND	0.91	0.86**
gi|110751139	Protein tyrosine phosphatase 99A CG2005-PB, isoform B [*Apis mellifera*]	Defasciculation of motor neuron axon	K.ETASGMILREVAVR.S	1**	0.96**	0.96*	1.01*
gi|118764591	Putative olfactory receptor 10R1 [*Sus scrofa*]	G-protein coupled receptor protein signaling pathway	K.MIGKTGFSVK.T	1	0.96	0.90	0.92
gi|194035919	Putative olfactory receptor 10R1 [*Sus scrofa*]	G-protein coupled receptor protein signaling pathway	K.MSTNVSDSVK.D	1	0.96	0.90	0.92
gi|55469457	Putative saitohin [*Pongo pygmaeus*]	G-protein coupled receptor protein signaling pathway	K.STKGLK.E	1	0.89**	0.92	0.96**
gi|149583995	Ran GTPase activating protein 1 [*Ornithorhynchus anatinus*]	Signal transduction	K.VSSVLK.D	1	0.89**	0.92	0.96**

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Signal transduction							
gi|20307022	RIKEN cDNA 2410091C18 gene [Mus musculus]	Protein amino acid dephosphorylation	K.VASLGPVEQR.T	1**	0.91	0.94	0.89
gi|126329915	Seven transmembrane helix receptor [*Monodelphis domestica*]	G-protein coupled receptor protein signaling pathway	K.ELTILSLISAKL.-	1	0.91	0.88	0.87
gi|126339999	Transducin beta-3-subunit [*Monodelphis domestica*]	signal transduction	R.VGTLSGHDNR.V	1**	0.91	0.94	0.89
gi|10190668	Vomeronasal type-1 receptor 1 [*Homo sapiens*]	G-protein coupled receptor protein signaling pathway	M.VGDTLK.L	1	0.89**	0.92	0.96**
gi|189235153	AGAP006107-PA [*Tribolium castaneum*]	intracellular signaling cascade	R.SVASRLDR.T	1**	0.88**	ND	0.91*
gi|189532342	F15D4.7 [*Danio rerio*]	Neuropeptide signaling pathway	K.VGESIK.K	1	0.89**	0.92	0.96
gi|221125263	PLC-deltaH [*Hydra magnipapillata*]	Intracellular signaling cascade	K.ICLNAESLQK.F	1	0.93**	1.01*	0.93*
gi|196013733	Predicted protein [*Trichoplax adhaerens*]	Neuropeptide signaling pathway	K.DLTVIEGTALSK.D	1	0.89	0.84	0.89
gi|148539556	Receptor for egg jelly 6 [*Strongylocentrotus purpuratus*]	Neuropeptide signaling pathway	R.VSSVIK.K	1	0.89**	0.92	0.96**
gi|2494282	RecName: Full=Delta-like protein 1; AltName: Full=Drosophila Delta homolog 1; Short=Delta1; Flags: Precursor	Notch signaling pathway	R.CQAGFSGR.Y	1	1.01*	0.90**	ND
gi|109502592	Retinitis pigmentosa RP1 protein-like [*Rattus norvegicus*]	Intracellular signaling cascade	R.GVSLCALPTR.V	1	0.92	1.04	0.89
Structural protein							
gi|213514450	Abl interactor 1 [*Salmo salar*]	Cytoplasm	K.EPKPKYTR.S	1	1.11	1.10	1.11
gi|189236667	AGAP005490-PA [*Tribolium castaneum*]	Membrane	R.SWYTEAMTSPK.D	1	1.04*	1.00	1.03*
gi|158289936	AGAP010396-PA [*Anopheles gambiae str. PEST*]	Microtubule-based movement	K.IEMCEAGSTLVK.V	1	0.92**	0.83**	0.97**

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Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Structural protein							
gi|224073847	Calmodulin regulated spectrin-associated protein 1 [*Taeniopygia guttata*]	Microtubule	R.GDPEVQNAAAR.V	1	0.96	0.94*	0.91**
gi|170045540	Chromosome-associated kinesin KIF4A [*Culex quinquefasciatus*]	Microtubule-based movement	K.ENMDTIEEVA.-	1	0.98	1.01**	1.00
gi|224079584	Circadian clock protein PER3 [*Taeniopygia guttata*]	Circadian rhythm	K.EELAEVHSWIR.T	1	0.93	1.49**	0.85*
gi|198430145	Dynamin [*Ciona intestinalis*]	Cell communication	K.MGRYPMLR.E	1	0.97	1.01*	0.95*
gi|149419178	EP37-L2 [*Ornithorhynchus anatinus*]	Cellular structure	R.SIPLCWK.L	1**	0.88**	ND	0.91
gi|159470179	Flagellar associated protein [*Chlamydomonas reinhardtii*]	Ciliary or flagellar motility	R.AAASMV.-	1	1.16	1.15	ND
gi|118095339	G elongation factor, mitochondrial 1 [*Gallus gallus*]	Mitochondrial translational elongation	K.GPVSGHK.I	1*	ND	1.32**	ND
gi|198451528	GA10594 [*Drosophila pseudoobscura pseudoobscura*]	Microtubule-based movement	K.KPTGAPGCTK.A	1	1.11	1.10	1.11
gi|125980578	GA10646 [*Drosophila pseudoobscura pseudoobscura*]	Microtubule-based movement	K.IKIINYR.Q	1	1.00*	1.05	1.05
gi|405715	Giantin [*Homo sapiens*]	Golgi organization	K.HKAEMEEK.T	1	1.11	1.10	1.11
gi|195397087	GJ16505 [*Drosophila virilis*]	Microtubule-based movement	R.LTRSD.-	1	ND	0.95**	0.88*
gi|195436925	GK18117 [*Drosophila willistoni*]	Integral to membrane	R.STTTEEK.Q	1*	0.76**	0.70*	ND
gi|195147524	GL19330 [*Drosophila persimilis*]	Cell proliferation	K.QGAAQIQAMGK.L	1	0.85	0.91	0.88
gi|134152381	Golga4 protein [*Mus musculus*]	Golgi apparatus	K.HAEQMEEK.E	1	1.11	1.10	1.11
gi|149445080	GPBP-interacting protein 130b [*Ornithorhynchus anatinus*]	Cytoplasm	R.ITIIQNASITPVK.S	1**	1.10*	1.15*	1.12*

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

124

Table 3.6 Differentially expressed proteins from testes of wild (group A and B according to the different protein patterns) and domesticated broodstock(14 and 18 months old) of *P. monodon* (cont.)

Accession no.	Protein identity	Function	Peptide	Intensity ratio			
				Wild-A	Wild-B	DB-14	DB-18
Structural protein							
gi|194215293	GRAM domain-containing protein 1A [*Equus caballus*]	Integral to membrane	K.AEKLALEEGGK.D	1	ND	1.15*	1.16*
gi|11611734	GREB1a [Homo sapiens]	Integral to membrane	K.EHMTK.Q	1**	1.21*	1.10*	ND
gi|149489715	KIAA1618 protein [*Ornithorhynchus anatinus*]	Cellular structure	K.ALMWTHLKK.L	1	ND	1.15*	1.16*
gi|194670654	Protein dispatched homolog 2 [*Bos taurus*]	Integral to membrane	R.DGTWKPASVQHHVVSVR.Q	1	0.97*	1.02**	ND
gi|115313664	Smith-Magenis syndrome chromosome region, candidate 7 [*Danio rerio*]	Cellular component	K.LLHKGIEGVVMK.Q	1	0.96	0.94	0.98
gi|149430167	Syntaphilin [*Ornithorhynchus anatinus*]	Cell junction	R.GAGAEQALNRDR.H	1	0.89	0.94	1.03
gi|198418863	Transmembrane protein 16E [*Ciona intestinalis*]	Integral to membrane	R.VNMPR.I	1	1.14**	1.07	1.15
gi|221106290	Uncharacterized protein C21orf63 [*Hydra magnipapillata*]	Integral to membrane	R.MFATVCSGK.T	1	1.11	1.10	1.11
gi|170571364	Zinc finger, C2H2 type family protein [*Brugia malayi*]	Multicellular organismal development	K.ESSSTVQTGNAER.F	1	0.91**	0.96	0.92

Typically, the average intensity in each group was calculated from 3 individuals but that calculated from 1 and 2 individuals are marked by one and two asterisks, respectively. ND = a particular protein that was not found in any individual within a group.

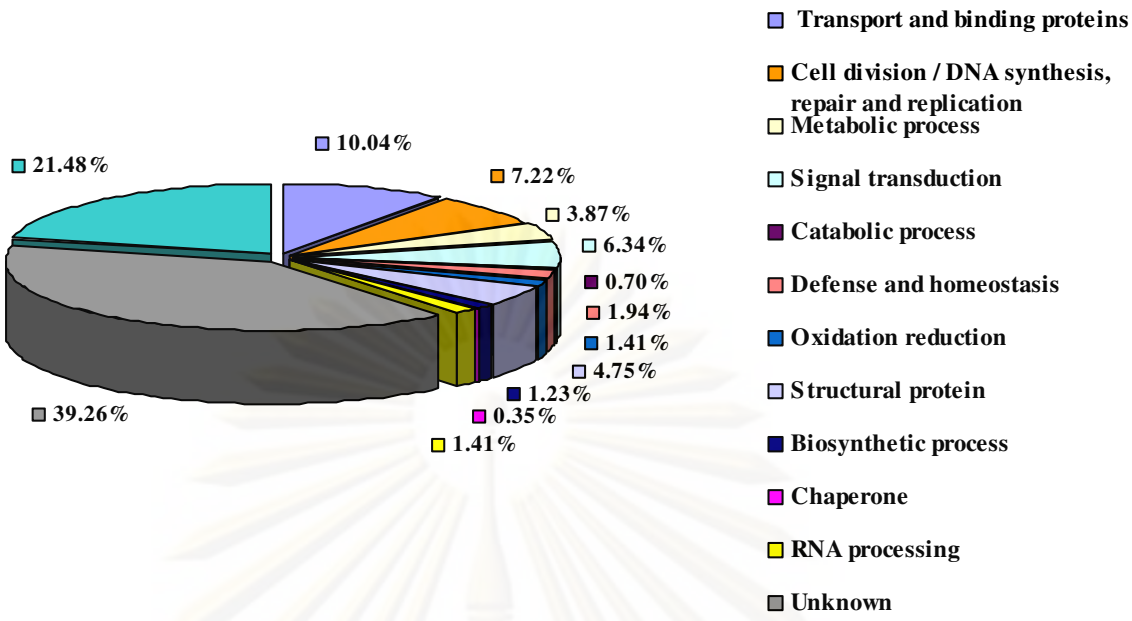


Figure 3.11 Function classification of 345 proteins identified form testes of wild (wild A and wild B) and domesticated (14 and 18 months old) *P. monodon* broodstock.

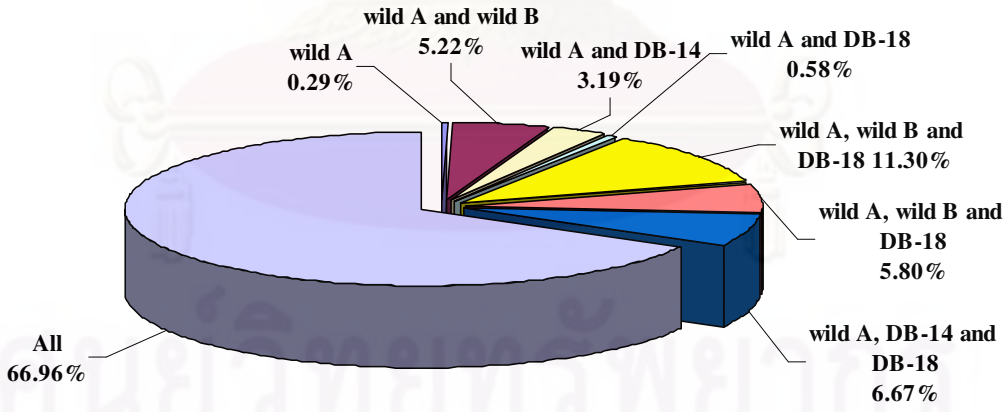


Figure 3.12 Groups of proteins classification of 345 proteins identified form testes of different groups of samples (wild A, wild B and domesticated 14-month-old and 18-month-old) of *P. monodon*.

Several different protein families were characterized. Reproduction-related proteins, for example, vasa-like protein, Ran GTPase activating protein 1 (RanGAP1) and seven transmembrane helix receptor etc., were identified.

Expression of *vasa-like DEAD-box proteins* has been shown in primodial germ cells (PGCs) of metazoans (Mochizuki et al., 2001). Vasa transcript or proteins are localized in germ granules or germ cells of various animals (Hay et al., 1988; Tanaka et al., 2000; Toyooka et al., 2000). Moreover, *vasa-like proteins* were shown to be expressed in adult germline stem cells in *Drosophila* (Hay et al., 1988), Hydra (Mochizuki et al., 2001) and *Crassostrea gigas* (Fabioux et al., 2004a).

RanGAP1 is the GTPase-activating protein for Ran, a small ras-like GTPase involved in regulating nucleocytoplasmic transport. In vertebrates, RanGAP1 is present in two forms: one that is cytoplasmic, and another that is concentrated at the cytoplasmic fibers of nuclear pore complexes (NPCs). The NPC-associated form of RanGAP1 is covalently modified by the small ubiquitin-like protein, SUMO-1, a member of a ubiquitin-related protein family. The nuclear localization signal, and the presence of nine leucine-rich nuclear export signal motifs, suggests that RanGAP1 may shuttle between the nucleus and the cytoplasm. (Matunis et al., 1998).

Recently, two totally distinct classes of putative membrane-bound progestin receptors have been reported in vertebrates: membrane progestin receptors (mPR, subtypes α, β, γ, also called progestin or adipoQ receptors; PAQR, VII, VIII and V, respectively; Zhu et al., 2003; Peluso et al., 2006) and progestin membrane receptor component (PGMRC subtypes 1 and 2; Mourot et al., 2006; Cahill, 2007; Thomas, 2008). The full length cDNA of *PGMRC1* (Leelatanawit et al., 2008; Preechaphol, 2008) was recently characterized in *P. monodon* but *mPR* has not been reported in any crustacean. The finding of a protein significantly matched seven transmembrane helix receptor of *Monodelphis domestica* open the possibility to characterize the novel nuclear progesterone receptor in *P. monodon*.

3.3 Isolation and characterization of the full length cDNA of genes expressed in testes of *P. monodon*

Several transcripts expressed in testes of *P. monodon* were further characterized by RACE-PCR. The full length cDNAs of four genes: *ubiquitin carboxyl-terminal hydrolase 14, ubiquitin carboxyl-terminal hydrolase 5, cyclin dependent kinase 17* and *proteasome alpha subunit*, were successfully isolated.

Cytoplasmic dynein 1 light intermediate chain 2

Two discrete fragments (approximately 550 and 180 bp in size) were obtained from 5'RACE-PCR (Figure 3.13, lane 1) whereas three discrete bands (approximately 2000, 1700 and 800 bp in size) were generated from 3'RACE-PCR (Figure 3.13, lane 2). A 550 bp fragment from the former was further characterized. Likewise, a 1700 bp fragment from the latter was excised from the gel, cloned and sequenced. Nucleotide sequences of the original EST and RACE-PCR (Figure 3.14) were assembled.

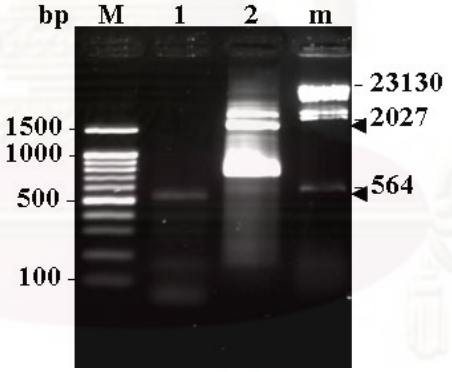


Figure 3.13 5' and 3'RACE-PCR products (lanes 1 and 2) of *cytoplasmic dynein 1 light intermediate chain 2*. Arrowheads indicate RACE-PCR products that were cloned and sequenced. Lanes M and m are a 100 bp DNA ladder and λ-*Hin*d III, respectively.

A.

ACGCGGGGGCGCTCAATGGG

B.

GAAAGGTCAGCCCTCTCGGGGCTTTCCGACCTCCGGAAAGAGAAGGCAGAGGAGAAGGAGAACCTTTGG

3'RACE-PCR

AAGAAAATCCTAGGCGAAGTACAATCGAGTGAGCGCAATAAATTACCATCGTGCAAGTCTGTTCTCGTC
CTGGGTGACAATGAGTCAGGGAAAACTACACTCATTGCCAAGCTACAGGGGAATGAGGACCCCAAAAAG
GGATCAGGCCTTGAATATGCCTACATCGATGTTAGGGATGAATACAGAGATGATCACACACGACTCAGT
GTCTGGGTTCTTGACGGTGACCCCAAACATGCTGAGCTTCTGGAGTTTGCAGTTAATGCAGACAATTTG
GAACACACATTGGTATTACTGACAGTGTCAATGACAGCCCCTTGGGGAATCATGGACCAGCTTCACACA

5'RACE-PCR

TGGGCCTCCACTCTCCAAGACCACATAGACAAGATCAACCTGGATCCTGACAAGTTTAAAGATCGGCAA
GATAAGATGGCTCGGTTATGGCAAGACTACGTAGAGCCTGGAGATGAACTAGAGGCTGGGTCACCCATG
AAGCGGTCATCAAGAAACCTGGAGAACGACGATGAACCTGTCTTGCCTCTCCCAGAAAATGTTCTTACC
AGAAATCTTGGACTTCACGTTATAGTTGTAGTTACTAAGACTGATTATATGTCAACTTTAGAAAAGGAC
TTTGATTATAAAGAAGAACACTTTGACTTCATTCAGCAGT

C.

CAATGAGTCAGGGAAAACTACACTCATTGCCAAGCTACAGGGGAATGAGGACCCCAAAAAGGGATCAGG
CCTTGAATATGCCTACATCGATGTTAGGGATGAATACAGAGATGATCACACACGACTCAGTGTCTGGGT
TCTTGACGGTGACCCCAAACATGCTGAGCTTCTGGAGTTTGCAGTTAATGCAGACAATTTGGAACACAC
ATTGGTATTACTGACAGTGTCAATGACAGCCCCTTGGGGAATCATGGACCAGCTTCACACATGGGCCTC
CACTCTCCAAGACCACATAGACAAGATCAACCTGGATCCTGACAAGTTTAAAGATCGGCAAGATAAGAT
GGCTCGGTTATGGCAAGACTACGTAGAGCCTGGAGATGAACTAGAGGCTGGGTCACCCATGAAGCGGTC
ATCAAGAAACCTGGAGAACGACGATGAACCTGTCTTGCCTCTCCCAGAAAATGTTCTTACCAGAAATCT
TGGACTTCACGTTATAGTTGTAGTTACTAAGACTGATTATATGTCAACTTTAGAAAAGGACTTTGATTA
TAAAGAAGAACACTTTGACTTCATTCAGCAGTCAATTCGAAAGTTCTGCCTTCAGTATGGTGCAGCTCT
TTTCTATACTTCAGTGAAGGAGGACAAGAACTGCGATCTGTTGTACAAGTATCTAGTCCATAAAATCTA
CAACTTCCCATTCAGAACCCCAGCACTTGTTGTTGAAAAAGATGCTGTGTTTATACCGGCAGGTTGGGA
CAACGATAAAAAGATTGCAATCCTTTATGAAAATATGCACACAATGAGTCCAGATGATTACTATACAGA
TGTCATTGTTAAGCCGCAAGTGGTTCGTAAGGCTGTTGCCCGGGAGGTGGAGGTTCAGGCTGAGGATGA
GCAAGCATTCTTAGCGCGGCACCAAGCACAGTTACAAGCAGGAGGCCCAGGTGCACCCAATGCTACACA
AGGTCGTCAAGAATCACCACTTAGACAGTCACCAGCTGTACAGAAGACCAGTGACCGACGGGTATCTTC
AACTGGAACACCAAATCAAATTGGTTCGCCAAAGAAGATTGAATCAACGAAGCCTGGAGTTGCTGGAGC
AAATGAAGGAGTCCTTGCAAACTTTTTCAATTTTTTTTTTAGTAAGAAAACTGGAACAAATGCCCCAGT
ACCTGGAGCCATAAAAACTAATGAGAAGGCTGCCATGCGATCAGATGCTGCTGCTGAGCTTGATCGTTT
AACGCGATCCAAGAAGCCAACAGCAGCAAATAGTTTTCCAGAAACTAACAACTCCTTTGAATGTTGACT
CCCAGTTGGCACCTCTACCGGCTGTGCTATGGAAAGGTGTTTTGGTGCCCCTGCCTGACAGTTTACACG
TACAGTTTTGTTAAGAGAATGGAAACAACTTCATGGCGTGTAGCGTTCATGGTGTCGGGGTTGGCTGCC
TGTCTGGTTGTTCCCTGTCGGAGAGGATTTGGCAAGGGATGTGAGACCTAATATGGGTAGCTTTTTCAT
TCCTTTGTATGATAAAATGCAATGTTAAGTCTACCTTTGAAAGGTACCATTTGGAAGGTATACAACTGT
AGCAGCTATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 3.14 Nucleotide sequences of 5' RACE-PCR (A), EST (B) and 3' RACE-PCR (C) fragments of *cytoplasmic dynein 1 light intermediate chain 2* of *P. monodon.* Primers for 5' and 3'RACE-PCR (underlined) were used for RT-PCR analysis of this gene.

The partial cDNA sequence of *P. monodon cytoplasmic dynein 1 light intermediate chain 2* was 1777 bp in length deduced to 510 amino acids (Figure 3.15). This sequence significantly matched *cytoplasmic dynein 1 light intermediate chain 2* of *Lepeophtheirus salmonis* (*E*-value = 3e-169).

```
ACGCGGGGGCGCTCAATGGGGAAAGGTCAGCCCTCTCGGGGCTTTCCGACCTCCGGAAAG 60
 A  G  A  L  N  G  E  R  S  A  L  S  G  L  S  D  L  R  K  E  20
AGAAGGCAGAGGAGAAGGAGAACCTTTGGAAGAAAATCCTAGGCGAAGTACAATCGAGTG 120
 K  A  E  E  K  E  N  L  W  K  K  I  L  G  E  V  Q  S  S  E  40
AGCGCAATAAATTACCATCGTGCAAGTCTGTTCTCGTCCTGGGTGACAATGAGTCAGGGA 180
 R  N  K  L  P  S  C  K  S  V  L  V  L  G  D  N  E  S  G  K  60
AAACTACACTCATTGCCAAGCTACAGGGGAATGAGGACCCCAAAAAGGGATCAGGCCTTG 240
 T  T  L  I  A  K  L  Q  G  N  E  D  P  K  K  G  S  G  L  E  80
AATATGCCTACATCGATGTTAGGGATGAATACAGAGATGATCACACACGACTCAGTGTCT 300
 Y  A  Y  I  D  V  R  D  E  Y  R  D  D  H  T  R  L  S  V  W  100
GGGTTCTTGACGGTGACCCCAAACATGCTGAGCTTCTGGAGTTTGCAGTTAATGCAGACA 360
 V  L  D  G  D  P  K  H  A  E  L  L  E  F  A  V  N  A  D  N  120
ATTTGGAACACACATTGGTATTACTGACAGTGTCAATGACAGCCCCTTGGGGAATCATGG 420
 L  E  H  T  L  V  L  L  T  V  S  M  T  A  P  W  G  I  M  D  140
ACCAGCTTCACACATGGGCCTCCACTCTCCAAGACCACATAGACAAGATCAACCTGGATC 480
 Q  L  H  T  W  A  S  T  L  Q  D  H  I  D  K  I  N  L  D  P  160
CTGACAAGTTTAAAGATCGGCAAGATAAGATGGCTCGGTTATGGCAAGACTACGTAGAGC 540
 D  K  F  K  D  R  Q  D  K  M  A  R  L  W  Q  D  Y  V  E  P  180
CTGGAGATGAACTAGAGGCTGGGTCACCCATGAAGCGGTCATCAAGAAACCTGGAGAACG 600
 G  D  E  L  E  A  G  S  P  M  K  R  S  S  R  N  L  E  N  D  200
ACGATGAACCTGTCTTGCCTCTCCCAGAAAATGTTCTTACCAGAAATCTTGGACTTCACG 660
 D  E  P  V  L  P  L  P  E  N  V  L  T  R  N  L  G  L  H  V  220
TTATAGTTGTAGTTACTAAGACTGATTATATGTCAACTTTAGAAAAGGACTTTGATTATA 720
 I  V  V  V  T  K  T  D  Y  M  S  T  L  E  K  D  F  D  Y  K  240
AAGAAGAACACTTTGACTTCATTCAGCAGTCAATTCGAAAGTTCTGCCTTCAGTATGGTG 780
 E  E  H  F  D  F  I  Q  Q  S  I  R  K  F  C  L  Q  Y  G  A  260
CAGCTCTTTTCTATACTTCAGTGAAGGAGGACAAGAACTGCGATCTGTTGTACAAGTATC 840
 A  L  F  Y  T  S  V  K  E  D  K  N  C  D  L  L  Y  K  Y  L  280
TAGTCCATAAAATCTACAACTTCCCATTCAGAACCCCAGCACTTGTTGTTGAAAAAGATG 900
 V  H  K  I  Y  N  F  P  F  R  T  P  A  L  V  V  E  K  D  A  300
CTGTGTTTATACCGGCAGGTTGGGACAACGATAAAAAGATTGCAATCCTTTATGAAAATA 960
 V  F  I  P  A  G  W  D  N  D  K  K  I  A  I  L  Y  E  N  M  320
TGCACACAATGAGTCCAGATGATTACTATACAGATGTCATTGTTAAGCCGCAAGTGGTTC 1020
 H  T  M  S  P  D  D  Y  Y  T  D  V  I  V  K  P  Q  V  V  R  340
GTAAGGCTGTTGCCCGGGAGGTGGAGGTTCAGGCTGAGGATGAGCAAGCATTCTTAGCGC 1080
 K  A  V  A  R  E  V  E  V  Q  A  E  D  E  Q  A  F  L  A  R  360
GGCACCAAGCACAGTTACAAGCAGGAGGCCCAGGTGCACCCAATGCTACACAAGGTCGTC 1140
 H  Q  A  Q  L  Q  A  G  G  P  G  A  P  N  A  T  Q  G  R  Q  380
AAGAATCACCACTTAGACAGTCACCAGCTGTACAGAAGACCAGTGACCGACGGGTATCTT 1200
 E  S  P  L  R  Q  S  P  A  V  Q  K  T  S  D  R  R  V  S  S  400
CAACTGGAACACCAAATCAAATTGGTTCGCCAAAGAAGATTGAATCAACGAAGCCTGGAG 1260
 T  G  T  P  N  Q  I  G  S  P  K  K  I  E  S  T  K  P  G  V  420
TTGCTGGAGCAAATGAAGGAGTCCTTGCAAACTTTTTCAATTTTTTTTTTAGTAAGAAAA 1320
 A  G  A  N  E  G  V  L  A  N  F  F  N  F  F  F  S  K  K  T  460
CTGGAACAAATGCCCCAGTACCTGGAGCCATAAAAACTAATGAGAAGGCTGCCATGCGAT 1380
 G  T  N  A  P  V  P  G  A  I  K  T  N  E  K  A  A  M  R  S  480
CAGATGCTGCTGCTGAGCTTGATCGTTTAACGCGATCCAAGAAGCCAACAGCAGCAAATA 1440
 D  A  A  A  E  L  D  R  L  T  R  S  K  K  P  T  A  A  N  S  500
GTTTTCCAGAAACTAACAACTCCTTTGAATGTTGACTCCCAGTTGGCACCTCTACCGGCT 1500
 F  P  E  T  N  N  S  F  E  C  *                              510
GTGCTATGGAAAGGTGTTTTGGTGCCCCTGCCTGACAGTTTACACGTACAGTTTTGTTAA 1560
GAGAATGGAAACAACTTCATGGCGTGTAGCGTTCATGGTGTCGGGGTTGGCTGCCTGTCT 1620
GGTTGTTCCCTGTCGGAGAGGATTTGGCAAGGGATGTGAGACCTAATATGGGTAGCTTTT 1680
TCATTCCTTTGTATGATAAAATGCAATGTTAAGTCTACCTTTGAAAGGTACCATTTGGAA 1720
GGTATACAACTGTAGCAGCTATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA      1777
```

Figure 3.15 Partial nucleotide and deduced amino sequences of *cytoplasmic dynein 1 light intermediate chain 2* of *P. monodon.* The stop codon (TGA) is illustrated in boldfaced and underlined. The poly A tail is boldfaced.

26S proteasome regulatory subunit S3

Two discrete fragments (approximately 400 and 300 bp in length) were obtained from 3' RACE-PCR of *26S proteasome regulatory subunit S3* (Figure 3.16, lane 1). Likewise, 5'RACE-PCR was further carried out and a 1600 bp fragment was obtained (Figure 3.16, lane 2). Nucleotide sequences of the original EST and RACE-PCR products (Figure 3.17) were assembled.

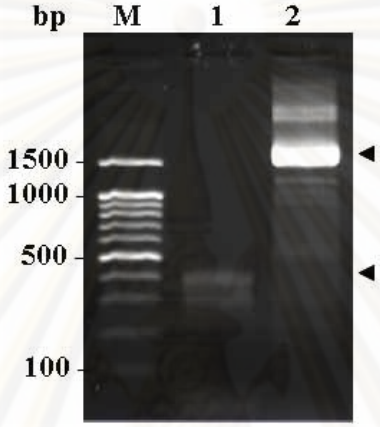


Figure 3.16 3' and 5'RACE-PCR product of *26S proteasome regulatory subunit S3* (lanes 1 and 2, respectively). Arrowheads indicate RACE-PCR products that were cloned and sequenced. Lane M is a 100 bp DNA ladder.

A.

ACGCGGGGGTTGTCATTTGAGAGGAAGCTTTTGTGCGTCGGTGATCCTCGTTTCGAATTTTGTCGGTGA
AAATGACGGTAGAGGCGATGGAAGTAACTACAAATGACAAGGAGAAAGAGAAGGAGAAGGAGACCGAGA
CAGAAAAGAAAGATCCGGATACATTATCGCT

B.

3'RACE-PCR

GGAAGATTTAAAGGAGCAGATACGCCTGGTTGAACGCAGCATTGTGAGCAAAGAACCTAGGTTTGTCCT
TCGTGTGCTGCGAGCACTCCCTGCTACAAGGAGGAAGCTTACCCCCAATGTACTCCGCTCCCTTGTGGC
TACTTACTATGGGCGACCAGAGAACAAGCAGGAGAGGGAGTCCATCCTCCAGTTCATTGAAGAGCCTAT
GGACACAGAGGCACCTCCACAAGCCAACTTGGGGCGTCAGCGTAGCCAGTTGGTGCTAAAGGTCGATGT
CTATATCCATCTCTTGGTTCTACTGCGACTTCTGGATACCAACAGCAATTCAGATGCTATTAAGTGCTC
AGATCTGTTGATGAACAAAGTGACATCAGTAAACAGGCGCACCCTTGACTTGCTGGCTGCACGCTGTTA
TTTTTATCACTCACGAGCTTATGAAGTTAACAGCAGACTGGATGAAATAAGAGGATTTTTGCACCAGCG
TCTGTGCCAGGCTACTCTCCGCAAGGACCATGAAGGACAGGCGGTCCTTATCAACTGTCTCCTGCGCAA
CTATCTCCACTACTCCCTCTACGACCAGGCACAGAAGCTGATTGTCAAGCTTGAGTTTCCTCAACAGGC
CAACAATAATGAGGTAGCACGGTACCAATACTACATGGGTCGCATCAAAGGTATCCAGTTGGAGTACTC
AGAGGCTCACAAGC

C.

ATCTCATTCAGGCTCTTCGCAAAGCTCCACAGCAGACAGCTGTAGGCTTCAGACAGTCCGTACAGAAGT
TGGCAGTAGTAGTGGAACTCCTGCTTGGTGATATCCCAGAGAGACAGATTTTCCGTCAGGCCATCATGC
GCAAAGCTCTGGCTCCTTATCTCCAGCTGACACAGGCTGTTAGGCTGGGTAATCAATGAATATAAGTCT
AAGTTCCTGGATGACCACACATTCATGCTCATCCTCCGTCTGCGCCACAACGTCATCAAGACAGGCTTA
CGAGCTATCTCGCTCTCCTACTCGCGCATCTCCCTAGCTGATGTTGCTGCCAAGTTGACTCTGGGCTCA
CGGGAAGATGCAGAGTTCATTGTAGCAAAAGCCATTAGGGATGGTGTCATTGAGGCTGTGATTGACCAT

```
GAGCATGGATATATGCAGAGCAAGGAGACTGTTGATGTATACTGCACTCGTGAACCACAGTCAGTGTAT
CATCAGCGCATTTCCTTCTGCTTGGATATCCACAACCAGTCGGTCAAGGCCATGCGCTACCCCCCCAAG
TCATACAACAAGGATCTTGAGAGTGCTGAGGAACGACGAGAACGGGAGCAGCAGGACCTGGAGTTGGCA
AAGGAGATGGCCGAGGAAGATGATGACAGTTTCACGTAGGCTGCCAGTTTCCCTCCTCTTTACCTCCGG
AGGGTAGAGCGCGCTTGAATGAGAGAGTACTGGTGTAATGTAGACAGAAAGAAATGTAATTGGTCAGGG
CTAAATGCTCTATCAGGAATATAAATTAGGCTTTCGAAATCCATTTCCGTATTACTTTGTCTCCCTTTT
TCCTTTCCATGTTGTAGGGATAATAACTTATAGTTTATAATTTTTGGAAAATGTGGTTTTATTTTCTTG
AAGTATCTTTGCTCTTGTTAATAAAGGAAACTTTGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

Figure 3.17 Nucleotide sequences of 5'RACE-PCR (A), EST (B) and 3'RACE-PCR (C) fragments of *26S proteasome regulatory subunit S3* of *P. monodon*. A primer for 3'RACE-PCR (underlined) was used as the forward primer for RT-PCR analysis of this gene.

The partial cDNA sequence of *P. monodon 26S proteasome regulatory subunit S3* was 1792 bp in length deduced to 495 amino acids (Figure 3.18). This sequence significantly matched *26S proteasome regulatory subunit S3* of *Nasonia vitripennis* (*E*-value = 2e-172).

```
TCCTCGTTTCGAATTTTGTCGGTGAAAATGACGGTAGAGGCGATGGAAGTAACTACAAAT 60
 S  S  F  R  I  L  S  V  K  M  T  V  E  A  M  E  V  T  T  N  20
GACAAGGAGAAAGAGAAGGAGAAGGAGACCGAGACAGAAAAGAAAGATCCGGATACATTA 120
 D  K  E  K  E  K  E  K  E  T  E  T  E  K  K  D  P  D  T  L  40
TCGCTGGAAGATTTAAAGGAGCAGATACGCCTGGTTGAACGCAGCATTGTGAGCAAAGAA 180
 S  L  E  D  L  K  E  Q  I  R  L  V  E  R  S  I  V  S  K  E  60
CCTAGGTTTGTCCTTCGTGTGCTGCGAGCACTCCCTGCTACAAGGAGGAAGCTTACCCCC 240
 P  R  F  V  L  R  V  L  R  A  L  P  A  T  R  R  K  L  T  P  80
AATGTGCTCCGCTCCCTTGTGGCTACTTACTATGGGCGACCAGAGAACAAGCAGGAGAGG 300
 N  V  L  R  S  L  V  A  T  Y  Y  G  R  P  E  N  K  Q  E  R  100
GAGTCCATCCTCCAGTTCATTGAAGAGCCTATGGACACAGAGGCACCTCCACAAGCCAAC 360
 E  S  I  L  Q  F  I  E  E  P  M  D  T  E  A  P  P  Q  A  N  120
TTGGGGCGTCAGCGTAGCCAGTTGGTGCTAGAGGTCGATGTCTATATCCATCTCTTGGTT 420
 L  G  R  Q  R  S  Q  L  V  L  E  V  D  V  Y  I  H  L  L  V  140
CTACTGCGACTTCTGGATACCAACAGCAATTCAGATGCTATTAAGTGCTCAGATCTGTTG 480
 L  L  R  L  L  D  T  N  S  N  S  D  A  I  K  C  S  D  L  L  160
ATGAACAAAGTGACATCAGTAAACAGGCGCACCCTTGACTTGCTGGCTGCACGCTGTTAT 540
 M  N  K  V  T  S  V  N  R  R  T  L  D  L  L  A  A  R  C  Y  180
TTTTATCACTCACGAGCTTATGAAGTTAACAGCAGACTGGATGAAATAAGAGGATTTTTG 600
 F  Y  H  S  R  A  Y  E  V  N  S  R  L  D  E  I  R  G  F  L  200
CACCAGCGTCTGTGCCAGGCTACTCTCCGCAAGGACCATGAAGGACAGGCTGTCCTTATC 660
 H  Q  R  L  C  Q  A  T  L  R  K  D  H  E  G  Q  A  V  L  I  220
AACTGTCTCCTGCGCAACTATCTCCACTACTCCCTCTACGACCAGGCACAGAAGCTGATT 720
 N  C  L  L  R  N  Y  L  H  Y  S  L  Y  D  Q  A  Q  K  L  I  240
GTCAAGCTTGAGTTTCCTCAACAGGCCAACAATAATGAGGTAGCACGGTACCACTACTAC 780
 V  K  L  E  F  P  Q  Q  A  N  N  N  E  V  A  R  Y  H  Y  Y  260
ATGGGTCGCATCAAGGGTATCCAGTTGGAGTACTCAGAGGCTCACAAGCATCTCATTCAG 840
 M  G  R  I  K  G  I  Q  L  E  Y  S  E  A  H  K  H  L  I  Q  280
GCTCTTCGCAAAGCTCCACAGCAGACAGCTGTAGGCTTCAGACAGTCCGTACAGAAGTTG 900
 A  L  R  K  A  P  Q  Q  T  A  V  G  F  R  Q  S  V  Q  K  L  300
GCAGTAGTAGTGGAACTCCTGCTTGGTGATATCCCAGAGAGACAGATTTTCCGTCAGGCC 960
 A  V  V  V  E  L  L  L  G  D  I  P  E  R  Q  I  F  R  Q  A  320
ATCATGCGCAAAGCTCTGGCTCCTTATCTCCAGCTGACACAGGCTGTTAGGCTGGGTAAC 1020
 I  M  R  K  A  L  A  P  Y  L  Q  L  T  Q  A  V  R  L  G  N  340
AATGAATATAAGTCTAAGTTCCTGGATGACCACACATTCATGCTCATCCTCCGTCTGCGC 1080
```

```
 N  E  Y  K  S  K  F  L  D  D  H  T  F  M  L  I  L  R  L  R  360
CACAACGTCATCAAGACAGGCTTACGAGCTATCTCGCTCTCCTACTCGCGCATCTCCCTA 1140
 H  N  V  I  K  T  G  L  R  A  I  S  L  S  Y  S  R  I  S  L  380
GCTGATGTTGCTGCCAAGTTGACTCTGGGCTCACGGGAAGATGCAGAGTTCATTGTAGCA 1200
 A  D  V  A  A  K  L  T  L  G  S  R  E  D  A  E  F  I  V  A  400
AAAGCCATTAGGGATGGTGTCATTGAGGCTGTGATTGACCATGAGCATGGATATATGCAG 1260
 K  A  I  R  D  G  V  I  E  A  V  I  D  H  E  H  G  Y  M  Q  420
AGCAAGGAGACTGTTGATGTATACTGCACTCGTGAACCACAGTCAGTGTATCATCAGCGC 1320
 S  K  E  T  V  D  V  Y  C  T  R  E  P  Q  S  V  Y  H  Q  R  440
ATTTCCTTCTGCTTGGATATCCACAACCAGTCGGTCAAGGCCATGCGCTACCCCCCCAAG 1380
 I  S  F  C  L  D  I  H  N  Q  S  V  K  A  M  R  Y  P  P  K  460
TCATACAACAAGGATCTTGAGAGTGCTGAGGAACGACGAGAACGGGAGCAGCAGGACCTG 1440
 S  Y  N  K  D  L  E  S  A  E  E  R  R  E  R  E  Q  Q  D  L  480
GAGTTGGCAAAGGAGATGGCCGAGGAAGATGATGACAGTTTCACGTAGGCTGCCAGTTTC 1500
 E  L  A  K  E  M  A  E  E  D  D  D  S  F  T  *              495
CCTCCTCTTTACCTCCGGAGGGTAGAGCGCGCTTGAATGAGAGAGTACTGGTGTAATGTA 1560
GACAGAAAGAAATGTAATTGGTCAGGGCTAAATGCTCTATCAGGAATATAAATTAGGCTT 1620
TCGAAATCCATTTCCGTATTACTTTGTCTCCCTTTTTCCTTTCCATGTTGTAGGGATAAT 1680
AACTTATAGTTTATAATTTTTGGAAAATGTGGTTTTATTTTCTTGAAGTATCTTTGCTCT 1740
TGTTAATAAAGGAAACTTTGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA          1792
```

Figure 3.18 Partial nucleotide and deduced amino sequences of *26S proteasome regulatory subunit S3* of *P. monodon.* The stop codon (TAG) is illustrated in boldfaced and underlined. The poly A tail is boldfaced.

Ubiquitin specific peptidase 14

Three fragments of approximately 1000, 1100 and 1700 bp in length were obtained from 3′RACE-PCR of *ubiquitin specific peptidase 14* (Figure 3.19). The largest fragment (1700 bp) was excised from the gel, cloned and sequenced. Nucleotide sequences of the original EST and the 3′RACE-PCR product (Figure 3.20) were assembled.

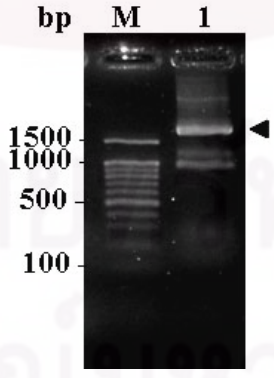


Figure 3.19 The primary 5'RACE-PCR product of *ubiquitin specific peptidase 14* (lane 1). An arrowhead indicates a RACE-PCR product that was cloned and sequenced. Lanes M is a 100 bp DNA ladder.

The full length cDNA of *ubiquitin specific peptidase 14* (*Ubi14*) of *P. monodon* was 2043 bp in length. The transcript contained an open reading frame (ORF) of 1524 bp corresponding to a putative protein of 507 amino acid and the 5'- and 3'UTRs of 124 and 274 bp (excluding the poly A tail), respectively (Figure 3.21).

The closest match to this transcript was *ubiquitin specific peptidase 14* of *Tribolium castaneum* (*E*-value = 8e-161). The predicted UBQ and UCH domains were found at positions 4-74 (*E*-value = 1.1e-09) and 103-428 (*E*-value = 2.4e-60) of the deduced Ubi14 protein (Figure 3.22). The calculated p*I* and MW of the deduced Ubi14 protein was 5.38 and 57.73 kDa, respectively.

A.

GTCTGAACCTCACGGTCAGCGGCGACCTCACCTCCTGACCACTTTATTCCCGGCGCTGCGTTCTCGACG
GGTATCGGGAGGAGCCACGCTGGGGAAAGTGATTCTTTGTTGAGGACCTTTCGCCATGACAGTCTTCAG
TGTGAATGTCAAATGGGGGAAGGAGATGTATCCAGGCATTGAACTGGATACTGCCGAACCCCCAATGGT
ATTCAAAGCTCAGTTGTTTGCCTTGACGGGAGTACAGCCTCACCGACAGAAGATCATGCTGAAAGGAGC

3'RACE-PCR

CACCATCAAGGATGAGACTTGGAATGGTGTCAAGCTGAAGGATGGGGCAACAGTTCTGATGATGGGGAG
CAAAGAGGAAGATGTGCCTGTAGAACCAACAGAGAAAACTGCTTTTGTTGAAGATATGACTGAAGCTGA
GAGGAACACTGCTTTGGAACTGCCTGTTGGCATTAAGAATCTGGGAAATACCTGTTATCTTAATGCAGT
TATCCAGTGCCTGAAAACAGTTCCTGAACTCCATTCTTCAGTTACGGAATTCAAGCCCAAGCCTCCTGG
GCGAGAAGGAGAGTCCTCCTCAGATCTTCTTACGCTTGCAGTAGATTCTGGATCTCTCCTTACACTGGC
TATCCAAGAATGCTAACGCACCATGGATCGTGGAACAACTGCCGTGCC

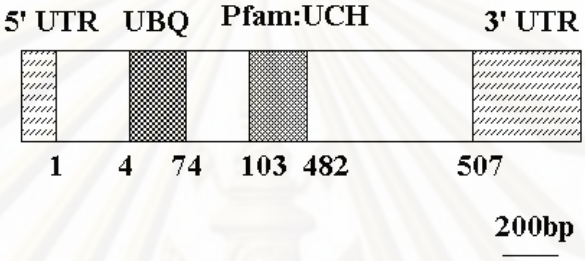
B.

CTTGGTCCTTGTCAACCTTTTCCGCACAACTTTTCCCAGATTTGCTGAACAAGGTGAGCAGGGGATGTA
CATGCAGCAGGATGCCTCTGAGTGCTGGAATGAACTTACCAGACTTTTGATGCAGGAAGTTCCTTCAAA
AGATGCAGAGAAGACGGACAAAAGGTTTGCATCATCCCTCATTGACCAATACTTCCCTGGAGAGTATTC
ATGTGAGTGGAAATGTATAGAAAGTGAAGAGGAAGGTGTGACACACAACACAGATAAATTCCAGCAACT
CATGTGCCATATCAACCAAGATGTAAAGTACCTACATACTGGTCTTACGGCCAAGATGGAGGAACACAT
TGACAAAAGATCACCTGTGTTAGACCGAGATGCAAAATATGTGAAAAAATCTAAAATTTCCAGATTACC
AGCCTATTTAACTGTAGTTATGGTCAGGTTTTTCTATAAAGAGAAAGGAGCAGTCAATGCCAAAATTCT
TAAAGATGTCAAATTCCCAATCAACCTAGATGTCTATGAACTTTGTACACCAGAACTACAGAAAAAGTT
ACAACCAATCAGAGAAAAATACAAGGACATGGATGATAGGAAGCTTGAAGAAGACAAAGCAAAGAGAAG
GGGTAAACCCATTTCGGAGGATAAGAAGCCAAAGACAAAGAAACTCCCATATTCCTTTGAGGATGATTT
AGGCAGCAACAACGGTGGTTACTACCAGCTTCAAGCTGTTTTAACTCACCAAGGTCGCTCCTCCTCATC
GGGTCACTATGTCAGCTGGGTGCGATGGCGTGGTGATGACTGGCTCAAATGTGATGATGATGAGGTCAC
GCCTGTCACTGAAGAAGAAATCCTGAGGCTTTCGGGAGGAGGTGATTGGCATTGTGCCTACATTTTGCT
GTATGGTCCACGAGTTCTAGAGGTTCTGGATGAAGATGAGAAGCTGCCAGCAGCTGCAGAGGTCAAGAT
GGAGACGGAGTGAGGGAGAAAGGGCATAGACCAGCTTTGCAATTTTAGTCATCTCAAATCAGGAGAATA
GATAATTTTTGAAATTGACAGGAAGAACAGCAGATTTTTCACAGATTTTCTTGGACCACCAAATGCTAA
TGTAATATAACACAAGTACTCACAAGGGAGATGAGTTTTTTCTTTTATGATAGAATGTATTCAACTTCC
ATACTTTTAGATTCATGTTTTAGGAGAATGCGGTGAGGAAGATTTGATATTTTGTTAATTTTATGGGTA
TGGGAGAGCATACTTTTAAATGGCAAGGGCATTCCCTTGTCCTCTAATTATTGTTTGTATGTTCATCCC
AAGCTGAGAAAATGGTTAAATTAAACTATTAAACTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 3.20 Nucleotide sequences of EST (A) and 3' RACE-PCR (B) of *ubiquitin specific peptidase 14* of *P. monodon*. A primer for 3'RACE-PCR (underlined) was used as the forward primer for RT-PCR analysis of this gene.

```
GTCTGAACCTCACGGTCAGCGGCGACCTCACCTCCTGACCACTTTATTCCCGGCGCTGCG  60
TTCTCGACGGGTATCGGGAGGAGCCACGCTGGGGAAAGTGATTCTTTGTTGAGGACCTTT  120
CGCCATGACAGTCTTCAGTGTGAATGTCAAATGGGGGAAGGAGATGTATCCAGGCATGAA  180
     M  T  V  F  S  V  N  V  K  W  G  K  E  M  Y  P  G  I  E   19
CTGGATACTGCCGAACCCCCAATGGTATTCAAAGCTCAGTTGTTTGCCTTGACGGGAGTA  240
  L  D  T  A  E  P  P  M  V  F  K  A  Q  L  F  A  L  T  G  V   39
CAGCCTCACCGACAGAAGATCATGCTGAAAGGAGCCACCATCAAGGATGAGACTTGGAAT  300
  Q  P  H  R  Q  K  I  M  L  K  G  A  T  I  K  D  E  T  W  N   59
GGTGTCAAGCTGAAGGATGGGGCAACAGTTCTGATGATGGGGAGCAAAGAGGAAGATGTG  360
  G  V  K  L  K  D  G  A  T  V  L  M  M  G  S  K  E  E  D  V   79
CCTGTAGAACCAACAGAGAAAACTGCTTTTGTTGAAGATATGACTGAAGCTGAGAGGAAC  420
  P  V  E  P  T  E  K  T  A  F  V  E  D  M  T  E  A  E  R  N   99
ACTGCTTTGGAACTGCCTGTTGGCATTAAGAATCTGGGAAATACCTGTTATCTTAATGCA  480
  T  A  L  E  L  P  V  G  I  K  N  L  G  N  T  C  Y  L  N  A  119
GTTATCCAGTGCCTGAAAACAGTTCCTGAACTCCATTCTTCAGTTACGGAATTCAAGCCC  540
  V  I  Q  C  L  K  T  V  P  E  L  H  S  S  V  T  E  F  K  P  139
AAGCCTCCTGGGCGAGAAGGAGAGTCCTCCTCAGATCTTCTTACGCTTGCAGTAGATTCT  600
  K  P  P  G  R  E  G  E  S  S  S  D  L  L  T  L  A  V  D  S  159
GGATCTCTCCTTACACTGGCTATCCAAGAATGCTACCGCACCATGGATCGTGGAACAACT  660
  G  S  L  L  T  L  A  I  Q  E  C  Y  R  T  M  D  R  G  T  T  179
GCCGTGCCCTTGGTCCTTGTCAACCTTTTCCGCACAACTTTTCCCAGATTTGCTGAACAA  720
  A  V  P  L  V  L  V  N  L  F  R  T  T  F  P  R  F  A  E  Q  199
GGTGAGCAGGGGATGTACATGCAGCAGGATGCCTCTGAGTGCTGGAATGAACTTACCAGA  780
  G  E  Q  G  M  Y  M  Q  Q  D  A  S  E  C  W  N  E  L  T  R  219
CTTTTGATGCAGGAAGTTCCTTCAAAAGATGCAGAGAAGACGGACAAAAGGTTTGCATCA  840
  L  L  M  Q  E  V  P  S  K  D  A  E  K  T  D  K  R  F  A  S  239
TCCCTCATTGACCAATACTTCCCTGGAGAGTATTCATGTGAGTGGAAATGTATAGAAAGT  900
  S  L  I  D  Q  Y  F  P  G  E  Y  S  C  E  W  K  C  I  E  S  259
GAAGAGGAAGGTGTGACACACAACACAGATAAATTCCAGCAACTCATGTGCCATATCAAC  960
  E  E  E  G  V  T  H  N  T  D  K  F  Q  Q  L  M  C  H  I  N  279
CAAGATGTAAAGTACCTACATACTGGTCTTACGGCCAAGATGGAGGAACACATTGACAAA  1020
  Q  D  V  K  Y  L  H  T  G  L  T  A  K  M  E  E  H  I  D  K  299
AGATCACCTGTGTTAGACCGAGATGCAAAATATGTGAAAAAATCTAAAATTTCCAGATTA  1080
  R  S  P  V  L  D  R  D  A  K  Y  V  K  K  S  K  I  S  R  L  319
CCAGCCTATTTAACTGTAGTTATGGTCAGGTTTTTCTATAAAGAGAAAGGAGCAGTCAAT  1140
  P  A  Y  L  T  V  V  M  V  R  F  F  Y  K  E  K  G  A  V  N  339
GCCAAAATTCTTAAAGATGTCAAATTCCCAATCAACCTAGATGTCTATGAACTTTGTACA  1200
  A  K  I  L  K  D  V  K  F  P  I  N  L  D  V  Y  E  L  C  T  359
CCAGAACTACAGAAAAAGTTACAACCAATCAGAGAAAAATACAAGGACATGGATGATAGG  1260
  P  E  L  Q  K  K  L  Q  P  I  R  E  K  Y  K  D  M  D  D  R  379
AAGCTTGAAGAAGACAAAGCAAAGAGAAGGGGTAAACCCATTTCGGAGGATAAGAAGCCA  1320
  K  L  E  E  D  K  A  K  R  R  G  K  P  I  S  E  D  K  K  P  399
AAGACAAAGAAACTCCCATATTCCTTTGAGGATGATTTAGGCAGCAACAACGGTGGTTAC  1380
  K  T  K  K  L  P  Y  S  F  E  D  D  L  G  S  N  N  G  G  Y  419
TACCAGCTTCAAGCTGTTTTAACTCACCAAGGTCGCTCCTCCTCATCGGGTCACTATGTC  1440
  Y  Q  L  Q  A  V  L  T  H  Q  G  R  S  S  S  S  G  H  Y  V  439
AGCTGGGTGCGATGGCGTGGTGATGACTGGCTCAAATGTGATGATGATGAGGTCACGCCT  1500
  S  W  V  R  W  R  G  D  D  W  L  K  C  D  D  D  E  V  T  P  459
GTCACTGAAGAAGAAATCCTGAGGCTTTCGGGAGGAGGTGATTGGCATTGTGCCTACATT  1560
  V  T  E  E  E  I  L  R  L  S  G  G  G  D  W  H  C  A  Y  I  479
TTGCTGTATGGTCCACGAGTTCTAGAGGTTCTGGATGAAGATGAGAAGCTGCCAGCAGCT  1620
  L  L  Y  G  P  R  V  L  E  V  L  D  E  D  E  K  L  P  A  A  499
GCAGAGGTCAAGATGGAGACGGAGTGAGGGAGAAAGGGCATAGACCAGCTTTGCAATTTT  1068
  A  E  V  K  M  E  T  E  *                                    507
AGTCATCTCAAATCAGGAGAATAGATAATTTTTGAAATTGACAGGAAGAACAGCAGATTT  1740
TTCACAGATTTTCTTGGACCACCAAATGCTAATGTAATATAACACAAGTACTCACAAGGG  1800
AGATGAGTTTTTTCTTTTATGATAGAATGTATTCAACTTCCATACTTTTAGATTCATGTT  1860
TTAGGAGAATGCGGTGAGGAAGATTTGATATTTTGTTAATTTTATGGGTATGGGAGAGCA  1920
TACTTTTAAATGGCAAGGGCATTCCCTTGTCCTCTAATTATTGTTTGTATGTTCATCCCA  1980
AGCTGAGAAAATGGTTAAATTAAACTATTAAACTCAAAAAAAAAAAAAAAAAAAAAAAAA  2040
AAAAAA                                                         2046
```

Figure 3.21 The full length cDNA and deduced amino acid sequences of *ubiquitin specific peptidase 14 of P. monodon* (2046 bp in length with an ORF of 1524 bp corresponding to a deduced polypeptide of 507 aa). The putative start (ATG) and stop (TGA) codons are underlined. The poly A tail is illustrated in boldface. The predicted UBQ (*E*-value = 1.1e-09, positions 4-74) and UCH (2.4e-60, positions 103-428) domains are highlighted.



Domain	Position	E-value
UBQ	4-74	1.15e-09
Pfam: UCH	103-482	5.30e-60

Figure 3.22 Diagram illustrating the deduced *ubiquitin specific peptidase 14* protein sequence of *P. monodon*. The predicted UBQ and UCH domains were found in this deduced protein.

Ubiquitinylation is an ATP-dependent process that involves the action of at least three enzymes: a ubiquitin-activating enzyme (UBE1), a ubiquitin-conjugating enzyme (UBE2), and a ubiquitin ligase (UBE3), which work sequentially in a cascade.

There are many different E3 ligases, which are responsible for the type of ubiquitin chain formed, the specificity of the target protein, and the regulation of the ubiquitinylation process (Hatakeyama and Nakayama, 2003). Accordingly, ubiquitinylation is an important regulatory tool that controls the concentration of key signaling proteins, such as those involved in cell cycle control, as well as removing misfolded, damaged and/or mutant proteins that could be harmful to the cells.

Ubiquitin carboxyl-terminal hydrolase 5

Several discrete bands were obtained from 5' and 3' RACE-PCR *ubiquitin carboxyl-terminal hydrolase 5*. A 950 bp fragment generated from 3' RACE-PCR was cloned and sequenced. In addition, semi-nested 5' RACE-PCR (using the original gene-specific primer + nested UPM primer) was further carried out and a 1500 bp fragment was further characterized (Figure 3.23). Nucleotide sequences of these fragments and EST (Figure 3.24) were assembled.

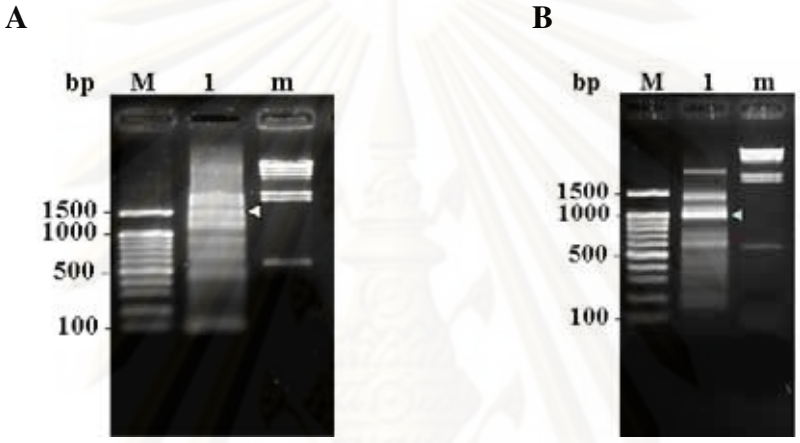


Figure 3.23 Semi-nested 5' (A) and 3' (B) RACE-PCR products of *ubiquitin carboxyl-terminal hydrolase 5*. Arrowheads indicate RACE-PCR products that were cloned and sequenced. Lanes M and m are a 100 bp DNA ladder and λ-*Hin*d III DNA marker, respectively.

The full length cDNA of *ubiquitin carboxyl-terminal hydrolase 5* of *P. monodon* was 3017 bp in length containing an ORF of 2442 bp corresponding to a putative protein of 813 amino acid with the 5' and 3' UTRs of 39 and 538 bp (excluding the poly A tail), respectively (Figure 3.25). The closest match to this transcript was *ubiquitin carboxyl-terminal hydrolase 5* of *Tribolium castaneum* (*E*-value = 0.0).

The predicted ZnF_UBP (*E*-value = 1.13e-17) and two UBA (*E*-values = 9.47e-07 and 2.48e-10) domains were found at positions 603-620, and 622-660 and 687-724 of the deduced protein (Figure 3.26). Four *N*-linked-glycosylation domain were found at positions 203-205, 350-352, 649-651 and 681-683. The calculated MW

and p*I* of the deduced *ubiquitin carboxyl-terminal hydrolase 5* was 90.80 kDa and 4.99, respectively.

A.

AAGCAGTGGTATCAACGCAGAGTACGCGGGATCGTAAGAATGGAGAAGTTGCGCGAACATTTTTCCAGG
ATTCGAGTTCCTAAAGGCGGGGACAAAGTGTACAAGGATGAGTGCATGTTCTCCCTCGATACCCCGGAG
AGTGAAACGGGACTTTATGTTTGCCTGAACAGTTTCTTTGGGTGGAGCAAGGAATACGTTGCCAAATAC
TCAGAACGTTCAGGAAATTGTGTATTCTTACACATTAAGAGAATCAAAAGAGAGCTTCCTCCAGAGAAA
GAACCAGAGCCCGACAAAAAAATTGCCCGTTTGGCAATTGGTGTCGAAGGTGGCTTCAACCCTGATGCA
AACAAAAAGAAATTTGAATACGAAGACACTAACTCTGTTGTGATTCTTCCAGCCTTTGATGTCATACCC
CTACCAAATCCTGATCTCCCAGAACTTGTACAGCAGAGCATTAATGGAGTACTGAAAGCAGAGTCTGCC
TTGCATTTGGCAGAAGTAGAGGCTGCTGCAGGTGCTTGGGACGGTGAGATACGGCAAGTCACCAAACAT
GCTGACAGCCTACAACAACTTGACAATGGGGTTAAGATACCACCAAGCGGTTGGAAATGTGAAGAATGT
GACAAGACGGATAACTTGTGGCTGAATCCCACAGACGGTAAGATTCTCTGCGGCAGACGGCTACTAGAT
GGATCAGGAGGAAACAACCATGCTGTGGAGTACTACCAGAAAACAAAATATCCACTAGCAGTGAAGCTT
GGGACCATCACCCAAGATGGCAATGCTGATGTCTACAGTTATGACGAAGACGACATGGTTTTGGATCCT
AACCTTGTTAAGCATTTAGCTCACTTTGGTATAAATGTCAAAGTTATGGAGAAGACAGAGAAGACCATG
TTGGAGCTAGAGATTGATTACAATCAGCGGGCTTGGGAATGGTCTCGCCTCACCGAGTCTGGGGCTAAA
CTCCTCCCCAAATTTGGCCCAGGTTACACAGGAATGAAAAATCTCGGCAACTCATGCTATGTGAATTCT
GTCATGCAAGTGCTGTTCACTGTACCGAACTTTGTGGAGCGGTACTTTGCAAATGGTACAAGTATCTTA
GAAGGTTACCAGGGAAATAATCCTGCTGATGACTTCAACATTCAGATGTTCAAACTTTCCCATGGCCTT
CTGTCTGGAAGGTACTCAGTTGCACCTCCCAACATCAACTTGGAAGAAGCTGTTGATACAGATGACCTG
CAGCCAGGTATATCTCCTGTCATGTTTAGAACCTTAGTTGGGAGAGGACATGCAGAATTCTCAACCAAG
AAGCAGCAAGATGCAATGGAGTTCCTTGAACACATCTTGAAGATGACCTCATGTAACTCAGCAGGAGTC
ACAGACCCAGGAAACTGTTTTAAATTTGAGGTTGAGGATAAGTTTGTATGCAGTGCCAGCAATAAGGTC
CGTTATGTGACAAGGCCTGACCAGTACCTACCGCTCC

B.

CAGTACCTGTTGATGAAGCTGTCAATAAAGAGGAGGTGGCAGCCTATCAAGCCCGCAAAGCAGAAGCCC
AAGCTTCACAGGTGGTCATGCAACCGGAGGAGCAGGTACGAGCAAAGATACCTTTCGATGTTTGTCTAT

3'RACE-PCR

CCAAGTTGGCTGCCCCTGAAGAAATCCTTGCATTTAGTTCAGCAGCAGAGAAAGAGGTTCCTATGCAGA
AGATTACACGCCTCAGGACATTCCCAGATTACCTAGTCATTCAGCTTGTCAAATTTGGCATCGGTCAAG
ATTGGGTTCCAATGAAGTATGATGTATCCATTGACATGCCAGAAGTTCTGGACTTGTCTGTTTTAAGAG
GGTTTGGACTGCAAGAAGGTGAAGAGGAGCTTCCAGAAACTACTGCTCCTCCTCCCAAAGAACCAGAAA
TAGATGCTGGTATTGTCCAACAATTAGCAGAAATGGGATTCCCGTGGGAGGCGTGTAGGAAAGCTGTTC
ACCTTACTGGGAACAATGGAACAGAGGCTGCCATGAACTGGGTCATGGAGCACATGGGTGACCCAGATT
TTGCTGACCCTCTTGTCATTAAGAGTGATACAAAAACAGGTAATGATACTTTCACTGCAAATGAAGAGG

5'RACE-PCR

GACTTGGAATGCTGATGTCCATGGGATTCACACGAGAGCAGGCAACTTTAGCTCTTAAGGAAACCAGCA
ATAATCTAGAACGTGCAGCAGATTGGATATTTTCAC

C.

ACCAACATGAGCTTGATTCTCTTTTAGCGGCACAGAGTGGTGCTGCTGCTCCTCCCCCACAAAAGCCAA
ACTACACTGATGGAGAACCTAAGTATGAGTTAACAGCATTTATCAGCCACATGGGAACCTCCATCTTTG
TTGGCCACTATGTCTGCCACATTAAGAAAGATGGAGAGTGGACAATCTTCAATGACAATAAAGTCTCTA
AATCCGCAGATCCACCCTTGGACCTTGGTTATATATACCTATATAAACGTGTAAATAATTAGGGACCAG
CGGTATGAATTTCAGAATCTGTAGCAGGAAGTAGGTAGTGGCCATTTTGAAAATTTCATTATTGTACCA
AGTGCAGACCTATACCAGAGGGAGGATGAAAGGAGGATGAATAGTAGATAGCTTAAGGAGTTTCACAAA
AGATTTTTTATGAGAATTTTTTTAGAGACTGAGTGTCATGTACCAATTACTTGGATGTTTTATGCATTT
CCCTTGTTTTTTCAGTTTAAAGATTTGTTTTACTTCATATTGGTTTTATGTTTTTGAGGCCAGGGTTTT
ATGCATTTTAATTTTACTTTTCCAAGTTTCATTTGTCCTCAAGACTGACGTGTTAACAAGAATTTATGC
TTTGTTTTCAGAGGAGAAAGAGCACTACACATCACATTATGCAAAGAAAAAGATTTATGACTTAACCAA
ATAAGAGGCCTTACATTTTTTTAAGAGATATGCAATATACAAAGTAAACAGAAATGTGATATATATAAA
TATATGGACATGTTCATGGG**AAAAAAAAAAAAAAAAAAAAAAAAAA**

Figure 3.24 Nucleotide sequence of Semi nested 5' RACE-PCR(A), EST (B) and 3' RACE-PCR (B) of *Ubiquitin carboxyl-terminal hydrolase 5* of *P. monodon*. A primer for RT-PCR (underlined) was used for 3' RACE-PCR of this gene.

```
AAGCAGTGGTATCAACGCAGAGTACGCGGGATCGTAAGAATGGAGAAGTTGCGCGAACAT 60
                                          M  E  K  L  R  E  H  7
TTTTCCAGGATTCGAGTTCCTAAAGGCGGGGACAAAGTGTACAAGGATGAGTGCATGTTC 120
 F  S  R  I  R  V  P  K  G  G  D  K  V  Y  K  D  E  C  M  F  27
TCCCTCGATACCCCGGAGAGTGAAACGGGACTTTATGTTTGCCTGAACAGTTTCTTTGGG 180
 S  L  D  T  P  E  S  E  T  G  L  Y  V  C  L  N  S  F  F  G  47
TGGAGCAAGGAATACGTTGCCAAATACTCAGAACGTTCAGGAAATTGTGTATTCTTACAC 240
 W  S  K  E  Y  V  A  K  Y  S  E  R  S  G  N  C  V  F  L  H  67
ATTAAGAGAATCAAAAGAGAGCTTCCTCCAGAGAAAGAACCAGAGCCCGACAAAAAAATT 300
 I  K  R  I  K  R  E  L  P  P  E  K  E  P  E  P  D  K  K  I  87
GCCCGTTTGGCAATTGGTGTCGAAGGTGGCTTCAACCCTGATGCAAACAAAAAGAAATTT 360
 A  R  L  A  I  G  V  E  G  G  F  N  P  D  A  N  K  K  K  F  107
GAATACGAAGACACTAACTCTGTTGTGATTCTTCCAGCCTTTGATGTCATACCCCTACCA 420
 E  Y  E  D  T  N  S  V  V  I  L  P  A  F  D  V  I  P  L  P  127
AATCCTGATCTCCCAGAACTTGTACAGCAGAGCATTAATGGAGTACTGAAAGCAGAGTCT 480
 N  P  D  L  P  E  L  V  Q  Q  S  I  N  G  V  L  K  A  E  S  147
GCCTTGCATTTGGCAGAAGTAGAGGCTGCTGCAGGTGCTTGGGACGGTGAGATACGGCAA 540
 A  L  H  L  A  E  V  E  A  A  A  G  A  W  D  G  E  I  R  Q  167
GTCACCAAACATGCTGACAGCCTACAACAACTTGACAATGGGGTTAAGATACCACCAAGC 600
 V  T  K  H  A  D  S  L  Q  Q  L  D  N  G  V  K  I  P  P  S  187
GGTTGGAAATGTGAAGAATGTGACAAGACGGATAACTTGTGGCTGAATCCCACAGACGGT 660
 G  W  K  C  E  E  C  D  K  T  D  N  L  W  L  N  P  T  D  G  207
AAGATTCTCTGCGGCAGACGGCTACTAGATGGATCAGGAGGAAACAACCATGCTGTGGAG 720
 K  I  L  C  G  R  R  L  L  D  G  S  G  G  N  N  H  A  V  E  227
TACTACCAGAAAACAAAATATCCACTAGCAGTGAAGCTTGGGACCATCACCCAAGATGGC 780
 Y  Y  Q  K  T  K  Y  P  L  A  V  K  L  G  T  I  T  Q  D  G  247
AATGCTGATGTCTACAGTTATGACGAAGACGACATGGTTTTGGATCCTAACCTTGTTAAG 840
 N  A  D  V  Y  S  Y  D  E  D  D  M  V  L  D  P  N  L  V  K  267
CATTTAGCTCACTTTGGTATAAATGTCAAAGTTATGGAGAAGACAGAGAAGACCATGTTG 900
 H  L  A  H  F  G  I  N  V  K  V  M  E  K  T  E  K  T  M  L  287
GAGCTAGAGATTGATTACAATCAGCGGGCTTGGGAATGGTCTCGCCTCACCGAGTCTGGG 960
 E  L  E  I  D  Y  N  Q  R  A  W  E  W  S  R  L  T  E  S  G  307
GCTAAACTCCTCCCCAAATTTGGCCCAGGTTACACAGGAATGAAAAATCTCGGCAACTCA 1020
 A  K  L  L  P  K  F  G  P  G  Y  T  G  M  K  N  L  G  N  S  327
TGCTATGTGAATTCTGTCATGCAAGTGCTGTTCACTGTACCGAACTTTGTGGAGCGGTAC 1080
 C  Y  V  N  S  V  M  Q  V  L  F  T  V  P  N  F  V  E  R  Y  347
TTTGCAAATGGTACAAGTATCTTAGAAGGTTACCAGGGAAATAATCCTGCTGATGACTTC 1140
 F  A  N  G  T  S  I  L  E  G  Y  Q  G  N  N  P  A  D  D  F  367
AACATTCAGATGTTCAAACTTTCCCATGGCCTTCTGTCTGGAAGGTACTCAGTTGCACCT 1200
 N  I  Q  M  F  K  L  S  H  G  L  L  S  G  R  Y  S  V  A  P  387
CCCAACATCAACTTGGAAGAAGCTGTTGATACAGATGACCTGCAGCCAGGTATATCTCCT 1260
 P  N  I  N  L  E  E  A  V  D  T  D  D  L  Q  P  G  I  S  P  407
GTCATGTTTAGAACCTTAGTTGGGAGAGGACATGCAGAATTCTCAACCAAGAAGCAGCAA 1320
 V  M  F  R  T  L  V  G  R  G  H  A  E  F  S  T  K  K  Q  Q  427
GATGCAATGGAGTTCCTTGAACACATCTTGAAGATGACCTCATGTAACTCAGCAGGAGTC 1380
 D  A  M  E  F  L  E  H  I  L  K  M  T  S  C  N  S  A  G  V  447
ACAGACCCAGGAAACTGTTTTAAATTTGAGGTTGAGGATAAGTTTGTATGCAGTGCCAGC 1440
 T  D  P  G  N  C  F  K  F  E  V  E  D  K  F  V  C  S  A  S  467
AATAAGGTCCGTTATGTGACAAGGCCTGACCAGTACCTACCGCTCCCAGTACCTGTTGAT 1500
 N  K  V  R  Y  V  T  R  P  D  Q  Y  L  P  L  P  V  P  V  D  487
GAAGCTGTCAATAAAGAGGAGGTGGCAGCCTATCAAGCCCGCAAAGCAGAAGCCCAAGCT 1560
 E  A  V  N  K  E  E  V  A  A  Y  Q  A  R  K  A  E  A  Q  A  507
TCACAGGTGGTCATGCAACCGGAGGAGCAGGTACGAGCAAAGATACCTTTCGATGTTTGT 1620
 S  Q  V  V  M  Q  P  E  E  Q  V  R  A  K  I  P  F  D  V  C  527
CTATCCAAGTTGGCTGCCCCTGAAGAAATCCTTGCATTTAGTTCAGCAGCAGAGAAAGAG 1680
 L  S  K  L  A  A  P  E  E  I  L  A  F  S  S  A  A  E  K  E  547
GTTCCTATGCAGAAGATTACACGCCTCAGGACATTCCCAGATTACCTAGTCATTCAGCTT 1740
 V  P  M  Q  K  I  T  R  L  R  T  F  P  D  Y  L  V  I  Q  L  567
GTCAAATTTGGCATCGGTCAAGATTGGGTTCCAATGAAGTATGATGTATCCATTGACATG 1800
 V  K  F  G  I  G  Q  D  W  V  P  M  K  Y  D  V  S  I  D  M  587
```

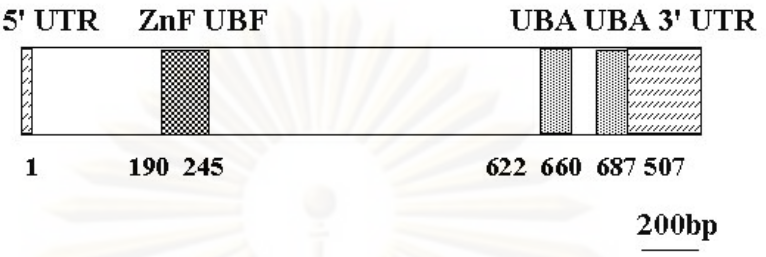
```
CCAGAAGTTCTGGACTTGTCTGTTTTAAGAGGGTTTGGACTGCAAGAAGGTGAAGAGGAG 1860
 P  E  V  L  D  L  S  V  L  R  G  F  G  L  Q  E  G  E  E  E  E  607
CTTCCAGAAACTACTGCTCCTCCTCCCAAAGAACCAGAAATAGATGCTGGTATTGTCCAA 1920
 L  P  E  T  T  A  P  P  P  K  E  P  E  I  D  A  G  I  V  Q  627
CAATTAGCAGAAATGGGATTCCCGTGGGAGGCGTGTAGGAAAGCTGTTCACCTTACTGGG 1980
 Q  L  A  E  M  G  F  P  W  E  A  C  R  K  A  V  H  L  T  G  647
AACAATGGAACAGAGGCTGCCATGAACTGGGTCATGGAGCACATGGGTGACCCAGATTTT 2040
 N  N  G  T  E  A  A  M  N  W  V  M  E  H  M  G  D  P  D  F  667
GCTGACCCTCTTGTCATTAAGAGTGATACAAAAACAGGTAATGATACTTTCACTGCAAAT 2100
 A  D  P  L  V  I  K  S  D  T  K  T  G  N  D  T  F  T  A  N  687
GAAGAGGGACTTGGAATGCTGATGTCCATGGGATTCACACGAGAGCAGGCAACTTTAGCT 2160
 E  E  G  L  G  M  L  M  S  M  G  F  T  R  E  Q  A  T  L  A  707
CTTAAGGAAACCAGCAATAATCTAGAACGTGCAGCAGATTGGATATTTTCACACCAACAT 2220
 L  K  E  T  S  N  N  L  E  R  A  A  D  W  I  F  S  H  Q  H  727
GAGCTTGATTCTCTTTTAGCGGCACAGAGTGGTGCTGCTGCTCCTCCCCCACAAAAGCCA 2280
 E  L  D  S  L  L  A  A  Q  S  G  A  A  A  P  P  P  Q  K  P  747
AACTACACTGATGGAGAACCTAAGTATGAGTTAACAGCATTTATCAGCCACATGGGAACC 2340
 N  Y  T  D  G  E  P  K  Y  E  L  T  A  F  I  S  H  M  G  T  767
TCCATCTTTGTTGGCCACTATGTCTGCCACATTAAGAAAGATGGAGAGTGGACAATCTTC 2400
 S  I  F  V  G  H  Y  V  C  H  I  K  K  D  G  E  W  T  I  F  787
AATGACAATAAAGTCTCTAAATCCGCAGATCCACCCTTGGACCTTGGTTATATATACCTA 2460
 N  D  N  K  V  S  K  S  A  D  P  P  L  D  L  G  Y  I  Y  L  807
TATAAACGTGTAAATAATTAGGACCAGCGGTATGAATTTCAGAATCTGTAGCAGGAAGT 2520
 Y  K  R  V  N  N  *                                          813
AGGTAGTGGCCATTTTGAAAATTTCATTATTGTACCAAGTGCAGACCTATACCAGAGGGA 2580
GGATGAAAGGAGGATGAATAGTAGATAGCTTAAGGAGTTTCACAAAAGATTTTTTATGAG 2640
AATTTTTTTAGAGACTGAGTGTCATGTACCAATTACTTGGATGTTTTATGCATTTCCCTT 2700
GTTTTTTCAGTTTAAAGATTTGTTTTACTTCATATTGGTTTTATGTTTTTGAGGCCAGGG 2760
TTTTATGCATTTTAATTTTACTTTTCCAAGTTTCATTTGTCCTCAAGACTGACGTGTTAA 2820
CAAGAATTTATGCTTTGTTTTCAGAGGAGAAAGAGCACTACACATCACATTATGCAAAGA 2880
AAAAGATTTATGACTTAACCAAATAAGAGGCCTTACATTTTTTTAAGAGATATGCAATAT 2940
ACAAAGTAAACAGAAATGTGATATATATAAATATATGGACATGTTCATGGGAAAAAAAAAA 3000
AAAAAAAAAAAAAAAAA                                            3017
```

Figure 3.25 The full length cDNA and deduced amino acid sequences of *ubiquitin carboxyl-terminal hydrolase 5 of P. monodon* (3017 bp in length with an ORF of 2442 bp corresponding to a deduced polypeptide of 813 aa). The putative start (ATG) and stop (TGA) codons are underlined. The poly A tail is illustrated in boldface. The predicted ZnF_UBP (1.13e-17) and two UBA (9.47e-07, 2.48e-10) domains (positions 603-620 of ZnF_UBP and 622-660 and 687-724) are highlighted.

Zinc finger (Znf) domains are relatively small protein motifs that bind one or more zinc atoms, and which usually contain multiple finger-like protrusions that make tandem contacts with their target molecules. Their binding properties depend on the amino acid sequence of the finger domains and of the linker between fingers, as well as on the higher-order structures and the number of fingers.

UBA domains are a commonly occurring sequence motif of approximately 45 amino acid residues that are found in diverse proteins involved in the

ubiquitin/proteasome pathway, DNA excision-repair, and cell signaling via protein kinases.



Domain	Position	E-value
ZnF UBP	190-245	1.13e-17
UBA	622-660	9.47e-07
UBA	687-724	2.48e-10

Figure 3.26 Diagram illustrating the deduced *ubiquitin carboxyl-terminal hydrolase 5* deduced protein of *P. monodon*. The predicted ZnF UBP and UBA domains were found in this deduced protein.

Cyclin dependent kinase 17 (Cdk17)

Several discrete bands were obtained from 5' and 3' RACE-PCR of *Cdk17*. A 1100 bp fragment generated from semi-nested 5' RACE-PCR (using the original gene-specific primers + nested UPM primer) was cloned and sequenced. In addition, 3' RACE-PCR was further carried out and a 1500 bp fragment was obtained (Figure 3.27). Nucleotide sequences of these fragments and EST (Figure 3.28) were assembled.

The full length cDNA of *Cdk17* of *P. monodon* was 1731 bp in length containing an ORF of 1470 bp corresponding to a putative protein of 489 amino acid with the 5' and 3' UTRs of 173 and 90 bp (excluding the poly A tail), respectively (Figure 3.29). The closest match to this transcript was *cyclin-dependent kinase 17* of *Xenopus laevis* (*E*-value=5e-178).

The N-linked-glycosylation domain was found at positions 38-40 and 476-478. The predicted S_TKc (*E*-value = 6.97e-96) domains was found at positions 160-

442 of the deduced Cdk17 protein of *P. monodon* (Figure 3.30). The calculated MW and p*I* of this deduced protein was 55.1567 kDa and 8.98, respectively.

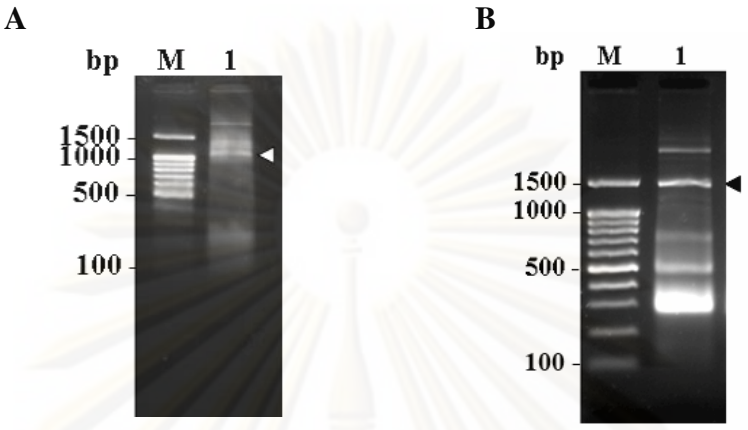


Figure 3.27 Semi-nested 5' (A) and 3' (B) RACE-PCR products of *Cdk17*. Arrowhead indicates RACE-PCR products that was cloned and sequenced. Lanes M is a 100 bp DNA ladder

A

ACGCGGGATAGCTAGAGAGGGGAAGAATCGGCAAAAATCAAACAAACGGAGGACTTGAGGGGAAAAGGA
AAGAAGAAGCTCTTTGCACAGATCACGTTTTACCCCCAGCCAGAGGCCCTTCCTCCGAACCCTTCCGGT
GGGCACTGAGTGTCGGCAGGGCAGCAGGATGTGTCATGAAACGCATCCGCCGGCGGTTGTCTCAGACCT
TCACCCGCTTCCACGATGGCAGCCTCACGGAGCTGGCCGAGCACCTCACCATCGACGAGAATGGGGGCA
TCAGAGAGAATGGATCGACCACAACGCCGACCTTCACGAGGATAAGTCGGCGGTTATCGCTCTCCAACT
CCCGCATCTTCAGCGACACTCACAAACAAGGTGTGGTGCACGAGGTACCAAGGATAGGGAGTGATGGCG
AGAGCGAAGAAGCTTCGGGAGCCAGTGATGAGGTCACCTCTCCTGTCAACGTCAAACTCAGGCAGAAAA
ATCGGCGTATCACACAAGAGGATATCAACAAACGCTTATCGCTGCCGGCGGACCTGCGGGTGCCCGACG
CCTTCCTCCAGAAGACGGCCATCAGCCCCGACGGACCTCTCAGCAGGGCCTCGCGGAGACAATCCCTCT
CGGAGATCGGCTTTGGGCGCATGGAAACCTACACCAAGCTCGATAAGTTAGGAGAGGGTACATATGCAA
CTGTGTACAAAGGACGGTCCCGACTAACAGACGCGTTAGTAGCCCTGAAAGAA

B

ATTCGTCTGGAGCATGAGGAAGGTGCACCATGTACAGCCATCAGGGAAGTGTCACTTCTCAAGGAACTT
AAACATGCAAATATTGTGACGTTACATGATATTGTTCATACAGACAAGAGTTTAACTCTTGTATTTGAA
TATTTAGACCGGGATCTCAAACAGTATATGGATGAATGTGGAGCACAACTATCAATGAATAATGTTAAG

5′ RACE-PCR

ATCTTCCTGTTCCAACTGCTTCGAGGGTTAGCCTATTGCCACCAGCGGCGAGTTCTCCACCGAGACCTC
AAGCCTCAGAACCTTCTCATCAATGACAAGGGAGAACTCAAGCTGGCAGACTTTGGCTTGGCACGTGC

3′ RACE-PCR

AAGTCAGTGCCAACCAAGACGTACAGTAACGAAGTAGTGACGTTGTGGTATCGGCCTCCGGATGTACTA
CTAGGCTCAACAGAATATTCAACACAAATTGATATGTGGGGAGTTGGATGCATCATGTACGAGATGATC
AGCGGACGACCACTCTTCCCAGGTGCCACAGTAGAAGATGAGCTCCANCTAATATTCCGAAACTTGGGC
AACCCTACAGAAG

C

CCACCTGGCCTGGCATTAGCACCAATGAAGATTTCAATCAGTATTCTTTCTCGTCTTATACTGGAGAAC
CCTTGCTAGCCCGAGCACCAAGACTTGCCCATGATTCAGCTGTCGGACTTTTAACGAAGTTCCTCTTGT
ATGAAGCTAAGAAGAGGATTTCTGCAGCAGCAGCACTAAAACACTCATTCTTCGAGTCTTTAGGTCACA
CAGTTCACACTCTCAAAGACCAGGATTCCATCTTCTCGTGTCCAGGAGTGATGCTAACACGCGACCACA
ACTACAAAGTAACGAATGGCAGTCAGGCCAAGACGCGGCGCCAGAGTATGCACTTCTGA

GCCCTGCCCAAGATTCCTTCCCATCAGTTTTGGTTTAAGACCCAGTCTGAAGACAGTCAATCGCAAAAA
AAAAAAAAAAAAAAAAAAA

Figure 3.28 Nucleotide sequence of semi-nested 5'RACE-PCR (A), EST (B) and 3'RACE-PCR (C) fragments of *Cdk17* of *P. monodon*.

```
ACGCGGGATAGCTAGAGAGGGGAAGAATCGGCAAAAATCAAACAAACGGAGGACTTGAGG 60
GGAAAAGGAAAGAAGAAGCTCTTTGCACAGATCACGTTTTACCCCCAGCCAGAGGCCCTT 120
CCTCCGAACCCTTCCGGTGGGCACTGAGTGTCGGCAGGGCAGCAGGATGTGTCATGAAAC 180
                                                    M  K   2
GCATCCGCCGGCGGTTGTCTCAGACCTTCACCCGCTTCCACGATGGCAGCCTCACGGAGC 240
R  I  R  R  R  L  S  Q  T  F  T  R  F  H  D  G  S  L  T  E  22
TGGCCGAGCACCTCACCATCGACGAGAATGGGGGCATCAGAGAGAATGGATCGACCACAA 300
L  A  E  H  L  T  I  D  E  N  G  G  I  R  E  N  G  S  T  T  42
CGCCGACCTTCACGAGGATAAGTCGGCGGTTATCGCTCTCCAACTCCCGCATCTTCAGCG 360
T  P  T  F  T  R  I  S  R  R  L  S  L  S  N  S  R  I  F  S  62
ACACTCACAAACAAGGTGTGGTGCACGAGGTACCAAGGATAGGGAGTGATGGCGAGAGCG 420
D  T  H  K  Q  G  V  V  H  E  V  P  R  I  G  S  D  G  E  S  82
AAGAAGCTTCGGGAGCCAGTGATGAGGTCACCTCTCCTGTCAACGTCAAACTCAGGCAGA 480
E  E  A  S  G  A  S  D  E  V  T  S  P  V  N  V  K  L  R  Q  102
AAAATCGGCGTATCACACAAGAGGATATCAACAAACGCTTATCGCTGCCGGCGGACCTGC 540
K  N  R  R  I  T  Q  E  D  I  N  K  R  L  S  L  P  A  D  L  122
GGGTGCCCGACGCCTTCCTCCAGAAGACGGCCATCAGCCCCGACGGACCTCTCAGCAGGG 600
R  V  P  D  A  F  L  Q  K  T  A  I  S  P  D  G  P  L  S  R  142
CCTCGCGGAGACAATCCCTCTCGGAGATCGGCTTTGGGCGCATGGAAACCTACACCAAGC 660
A  S  R  R  Q  S  L  S  E  I  G  F  G  R  M  E  T  Y  T  K  162
TCGATAAGTTAGGAGAGGGTACATATGCAACTGTGTACAAAGGACGGTCCCGACTAACAG 720
L  D  K  L  G  E  G  T  Y  A  T  V  Y  K  G  R  S  R  L  T  182
ACGCGTTAGTAGCCCTGAAAGAAATTCGTCTGGAGCATGAGGAAGGTGCACCATGTACAG 780
D  A  L  V  A  L  K  E  I  R  L  E  H  E  E  G  A  P  C  T  202
CCATCAGGGAAGTGTCACTTCTCAAGGAACTTAAACATGCAAATATTGTGACGTTACATG 840
A  I  R  E  V  S  L  L  K  E  L  K  H  A  N  I  V  T  L  H  222
ATATTGTTCATACAGACAAGAGTTTAACTCTTGTATTTGAATATTTAGACCGGGATCTCA 900
D  I  V  H  T  D  K  S  L  T  L  V  F  E  Y  L  D  R  D  L  242
AACAGTATATGGATGAATGTGGAGCACAACTATCAATGAATAATGTTAAGATCTTCCTGT 960
K  Q  Y  M  D  E  C  G  A  Q  L  S  M  N  N  V  K  I  F  L  262
TCCAACTGCTTCGAGGGTTAGCCTATTGCCACCAGCGGCGAGTTCTCCACCGAGACCTCA 1020
F  Q  L  L  R  G  L  A  Y  C  H  Q  R  R  V  L  H  R  D  L  282
AGCCTCAGAACCTTCTCATCAATGACAAGGGAGAACTCAAGCTGGCAGACTTTGGCTTGG 1080
K  P  Q  N  L  L  I  N  D  K  G  E  L  K  L  A  D  F  G  L  302
CACGTGCCAAGTCAGTGCCAACCAAGACGTACAGTAACGAAGTAGTGACGTTGTGGTATC 1140
A  R  A  K  S  V  P  T  K  T  Y  S  N  E  V  V  T  L  W  Y  322
GGCCTCCGGATGTACTACTAGGCTCAACAGAATATTCAACACAAATTGATATGTGGGGAG 1200
R  P  P  D  V  L  L  G  S  T  E  Y  S  T  Q  I  D  M  W  G  342
TTGGATGCATCATGTACGAGATGATCAGCGGACGACCACTCTTCCCAGGTGCCACAGTCG 1260
V  G  C  I  M  Y  E  M  I  S  G  R  P  L  F  P  G  A  T  V  362
AAGATGAGCTCCACCTAATATTCCGAACCTTGGGCACCCCTACAGAAGCCACCTGGCCTG 1320
E  D  E  L  H  L  I  F  R  T  L  G  T  P  T  E  A  T  W  P  382
GCATTAGCACCAATGAAGATTTCAATCAGTATTCTTTCTCGTCTTATACTGGAGAACCCT 1380
G  I  S  T  N  E  D  F  N  Q  Y  S  F  S  S  Y  T  G  E  P  402
TGCTAGCCCGAGCACCAAGACTTGCCCATGATTCAGCTGTCGGACTTTTAACGAAGTTCC 1440
L  L  A  R  A  P  R  L  A  H  D  S  A  V  G  L  L  T  K  F  422
TCTTGTATGAAGCTAAGAAGAGGATTTCTGCAGCAGCAGCACTAAAACACTCATTCTTCG 1500
L  L  Y  E  A  K  K  R  I  S  A  A  A  A  L  K  H  S  F  F  442
AGTCTTTAGGTCACACAGTTCACACTCTCAAAGACCAGGATTCCATCTTCTCGTGTCCAG 1560
E  S  L  G  H  T  V  H  T  L  K  D  Q  D  S  I  F  S  C  P  462
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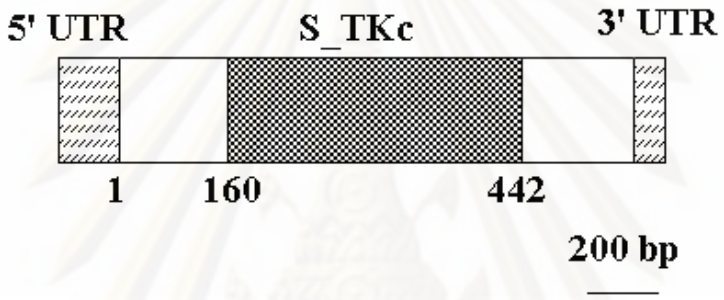
```
GAGTGATGCTAACACGCGACCACAACTACAAAGTAACGAATGGCAGTCAGGCCAAGACGC 1620
 G  V  M  L  T  R  D  H  N  Y  K  V  T  N  G  S  Q  A  K  T  482
GGCGCCAGAGTATGCACTTCTGAGCCCTGCCCAAGATTCCTTCCCATCAGTTTTGGTTTA 1680
 R  R  Q  S  M  H  F  *                                       489
AGACCCAGTCTGAAGACAGTCAATCGCAAAAAAAAAAAAAAAAAAAAAAAA          1731
```

Figure 3.29 The full length cDNA sequences of *Cdk17 of P. monodon* (1731 bp in length with an ORF of 1470 bp corresponding to a deduced polypeptide of 489 aa). The putative start (ATG) and stop (TGA) codons are underlined. The poly A tail is illustrated in boldface. The predicted S_TKc (6.97e-96) domains (positions 160-442 of S_TKc) are highlighted.



Domain	Position	E-value
S_TKc	160-442	6.97e-96

Figure 3.30 Diagram illustrating the deduced Cdk17 protein of *P. monodon*. The predicted S_TKc domain was found in this deduced protein.

Protein kinases are a group of enzymes that possess a catalytic subunit which transfers the gamma phosphate from nucleotide triphosphates (often ATP) to one or more amino acid residues in a protein substrate side chain, resulting in a conformational change affecting protein functions. The enzymes fall into two broad classes, characterized with respect to substrate specificity: serine/threonine specific and tyrosine specific kinases.

Proteasome alpha subunit

Two fragments of approximately 900 and 1200 bp in length were obtained from 3′RACE-PCR *proteasome alpha subunit* (Figure 3.31). The largest fragment (1200 bp) was excised from the gel, cloned and sequenced. Nucleotide sequences of the original EST and 3′RACE-PCR fragments (Figure 3.32) were assembled.

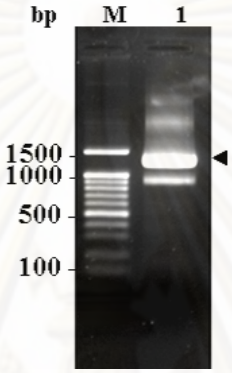


Figure 3.31 The primary 3'RACE-PCR product of *proteasome alpha subunit.* An arrowhead indicates a RACE-PCR product that was cloned and sequenced. Lane M is a 100 bp DNA ladder.

The full length cDNA of *proteasome alpha subunit* of *P. monodon* was 1394 bp in length. The transcript contained an ORF of 765 bp corresponding to a putative protein of 254 amino acid and the 5' and 3' UTRs of 70 and 561 bp (excluding the poly A tail), respectively (Figure 3.33). The closest match to this transcript was *proteasome alpha subunit* of *Ixodes scapularis* (*E*-value = 4e-104).

The predicted Proteasome_A_N (*E*-value = 5.05e-07) and Proteasome (*E*-value = 1.80e-63) domains were found at positions 8-30 and 31-216 of the deduced protein, respectively (Figure 3.34). The calculated MW and p*I* of the deduced proteasome alpha subunit, protein was 27.8617 kDa and 5.27, respectively.

The *proteasome* is a eukaryotic and archaeal multicatalytic proteinase complex that seems to be involved in an ATP/ubiquitin-dependent nonlysosomal proteolytic pathway. ATP-dependent protease complexes are present in all three kingdoms of life, where they rid the cell of misfolded or damaged proteins and control the level of certain regulatory proteins.

A

AGTCAGTCCCGGCGGCGTCGTGACAGAGATCCATTTGTCGTGAAAAGGCTTCTGTGGCATTTAATACAC
CATGAGTTCCATCGGTACTGGGTACGATCTTTCAGCCTCACAATTTTCGCCTGATGGCCGAGTGTTCCA
3′ RACE-PCR
AGTAGAGTATGCCCAAAAAGCAGTCGAGAACAGTGGAACTGCTGTTGGCTTACGCTGCAAAGATGGTGT
TGTGTTTGCTGTAGAGAAGATCATTACCTCAAAACTTTATGAACCTGGGGCAAATAAGCGCATCTTCAC
TGTGGACACACATGCAGGAATTGCCTGCGCTGGATTATTAGCTGATGCTCGTGCTATCGTTGATGTAGC
CAGAATTGAAGCTTCTAATTACCGTGCTGAGTATGGAATGCCCATCCCTTGCATGCTGTTGGCAGAACG
AGTGAGCACCTACCTTCATGCCTATACCCTCTACTCTGCTGTACGACCATATGGCTGCTCTGTAATGAT
TGGTGCCTTTGATAAGGATGGACCACAGCTGTACCTCACAGATCCAGCTGGCATGTGTAATGGTTTCTT
TGGATGTGCAGTAAGCAAGGCTAAGCAGAATGCTAAGACAGAAATCGAGAAGCTGAAACTCCAAGGATT
TAAGCTG

B

TAAGGAATTAGTGAAAGAAGCTGCAAAAATCATCTACCTTGTCCACGATGAAGTAAAAGACCGTCACTT
CGAGCTGGAACTGTCTTGGGTGTGCAAGGAGTCTGGGGGCCGTCACCAACGCGTGCCGAAGGACCTGTA
TGAGGAGGCGGAAAGATATGCCAAGGCAGCCCTGGTAGAGGACTCGTCAGATGAGGACGAGGAGATGTA
GAGGTGAATGAGTGGGACTTACTAGAGGCAGAAGGAAGTAAAACAAATGGTGTATTTTTGCCTTTCTCT
TTTTTTTCTTTTTTCTTTTTAATTATTTGTGAAGACTTGGGTGATATCAAACAAGATTTCTGACAAGA
TGGAGTCTTGGGTACGTAATTGTTTGGTCGTTGCTGAAATAAACAGTTACCCCATACATAAAATATTTT
GTATTAACACATCCCCCTTTTATAAGAAAATCTTGTGAATTTGAAGATCGTTTTGAATGATATACCCTC
ATAGAATATGTCTTGCATTTGGGATCATAATCCTTGCGGGAGAGTGATCTATCAATTGAATTGTTCAGA
ATCATCCATGAATGGCATTTGATTTCCTGGTTGCATGAAATTAGATGTTTTTATGGCCTATTGATCTTC
AAAAGGAATTGATATTTCAAACAGCAGGAATTCTCCACTGGTTATAAGGATAGGGAGTCAGATCATGCT
AAGATAAAACACTATTTTCTGTTTGTGTGAATGAATAAATTTAATAAGTAAAAAAAAAAAAAAAAAAA
AAAAAAAA

Figure 3.32 Nucleotide sequences of EST (A) and 3' RACE-PCR (B) fragments of *proteasome alpha subunit, putative* of *P. monodon*. The 3' RACE-PCR primer (underlined) was used for RT-PCR analysis of this gene.

AGTCAGTCCCGGCGGCGTCGTGACAGAGATCCATTTGTCGTGAAAAGGCTTCTGTGGCAT 60
TTAATACACCATGAGTTCCATCGGTACTGGGTACGATCTTTCAGCCTCACAATTTTCGCC 120
M S S I G T G Y D L S A S Q F S P 17
TGATGGCCGAGTGTTCCAAGTAGAGTATGCCCAAAAAGCAGTCGAGAACAGTGGAACTGC 180
D G R V F Q V E Y A Q K A V E N S G T A 37
TGTTGGCTTACGCTGCAAAGATGGTGTTGTGTTTGCTGTAGAGAAGATCATTACCTCAAA 240
V G L R C K D G V V F A V E K I I T S K 57
ACTTTATGAACCTGGGGCAAATAAGCGCATCTTCACTGTGGACACACATGCAGGAATTGC 300
L Y E P G A N K R I F T V D T H A G I A 77
CTGCGCTGGATTATTAGCTGATGCTCGTGCTATCGTTGATGTAGCCAGAATTGAAGCTTC 360
C A G L L A D A R A I V D V A R I E A S 97
TAATTACGGTGCTGAGTATGGAATGCCCATCCCTTGCATGCTGTTGGCAGAACGAGTGAG 420
N Y G A E Y G M P I P C M L L A E R V S 117
CACCTACCTTCATGCCTATACCCTCTACTCTGCTGTACGACCATATGGCTGCTCTGTAAT 480
T Y L H A Y T L Y S A V R P Y G C S V M 137
GATTGGTGCCTTTGATAAGGATGGACCACAGCTGTACCTCACAGATCCAGCTGGCATGTG 540
I G A F D K D G P Q L Y L T D P A G M C 157
TAATGGTTTCTTTGGATGTGCAGTAGGCAAGGCTAAGCAGAATGCTAAGACAGAAATCGA 600
N G F F G C A V G K A K Q N A K T E I E 177
GAAGCTGAAACTCAAAGATTTAAGCTGTAAGGAATTAGTGAAAGAAGCTGCAAAAATCAT 660
K L K L K D L S C K E L V K E A A K I I 197
CTACCTTGTCCACGATGAAGTAAAAGACCGTCACTTCGAGCTGGAACTGTCTTGGGTGTG 720
Y L V H D E V K D R H F E L E L S W V C 217
CAAGGAGTCTGGGGGCCGTCACCAACGCGTGCCGAAGGACCTGTATGAGGAGGCGGAAAG 780
K E S G G R H Q R V P K D L Y E E A E R 237
ATATGCCAAGGCAGCCCTGGTAGAGGACTCGTCAGATGAGGACGAGGAGATGTAGAGGTG 840

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 Y  A  K  A  A  L  V  E  D  S  S  D  E  D  E  E  M  *          254
AATGAGTGGGACTTACTAGAGGCAGAAGGAAGTAAAACAAATGGTGTATTTTTGCCTTTC 900
TCTTTTTTTTTCTTTTTTCTTTTTAATTATTTGTGAAGACTTGGGTGATATCAAACAAGA 960
TTTCTGACAAGATGGAGTCTTGGGTACGTAATTGTTTGGTCGTTGCTGAAATAAACAGTT 1020
ACCCCATACATAAAATATTTTGTATTAACACATCCCCCTTTTATAAGAAAATCTTGTGAA 1080
TTTGAAGATCGTTTTGAATGATATACCCTCATAGAATATGTCTTGCATTTGGGATCATAA 1140
TCCTTGCGGGAGAGTGATCTATCAATTGAATTGTTCAGAATCATCCATGAATGGCATTTG 1200
ATTTCCTGGTTGCATGAAATTAGATGTTTTTATGGCCTATTGATCTTCAAAAGGAATTGA 1260
TATTTCAAACAGCAGGAATTCTCCACTGGTTATAAGGATAGGGAGTCAGATCATGCTAAG 1320
ATAAAACACTATTTTCTGTTTGTGTGAATGAATAAATTTAATAAGTAAAAAAAAAAAAAA 1380
AAAAAAAAAAAAAA                                               1394
```
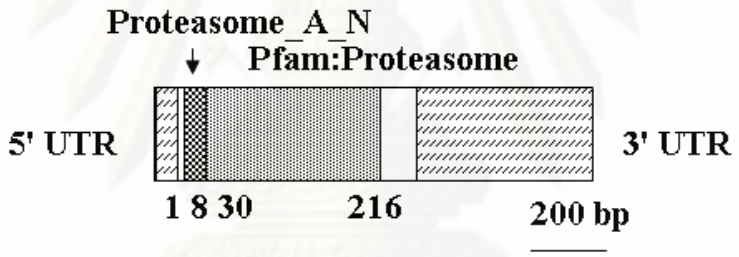

Figure 3.33 The full length cDNA sequences of *proteasome alpha subunit, putative* of *P. monodon* (1394 bp in length with an ORF of 765 bp corresponding to a deduced polypeptide of 254 aa). The putative start (ATG) and stop (TAG) codons are underlined. The poly A tail is illustrated in boldface. The predicted Proteasome_A_N (5.05e-07) and Proteasome (1.80e-63) domains (positions 8-30 of Proteasome_A_N and 31-216 of Proteasome) are highlighted.



Domain	Position	E-value
Proteasome_A_N	8-30	5.05e-07
Pfam:Proteasome	31-216	1.80e-63

Figure 3.34 Diagram illustrating the deduced proteasome alpha subunit protein of *P. monodon*. The Proteasome A_N and Proteasome domains were found in the deduced protein of this transcript.

3.4 RT-PCR and tissue distribution analysis of various gene homologues

Testicular total RNA revealed several discrete bands along with smeared high-to-low molecular weight RNA (Figure 3.35). The ratios of purified total RNA were 1.7-2.0 implying that the quality of extracted total RNA was acceptable for further applications. The first cDNA synthesized from DNase I-treated total RNA covered the large product sizes (Figure 3.36).

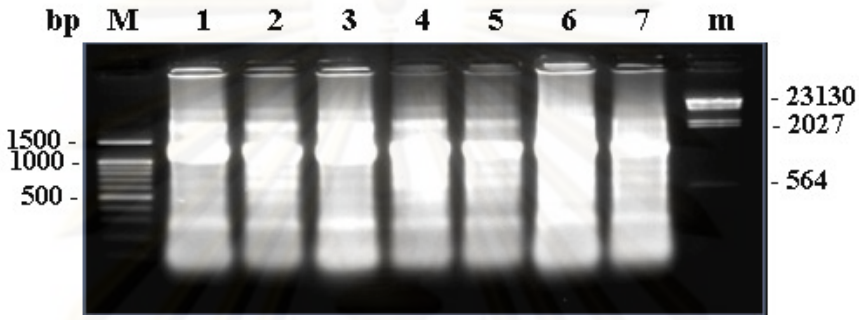


Figure 3.35 A 1.2% ethidium bromide-stained agarose gel showing the quality of total RNA extracted from testes of male *P. monodon* broodstock. Lanes M and m = a 100 bp DNA ladder and λ-*Hin*d III, respectively. Lanes 1-7 = total RNA from different individuals of *P. monodon*.

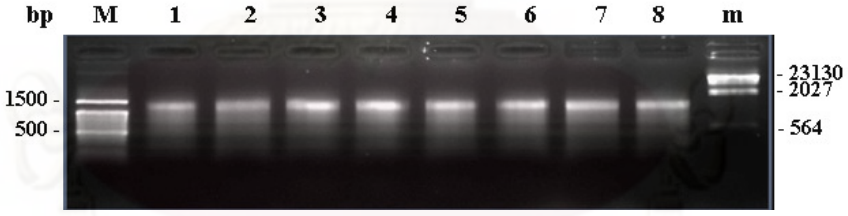


Figure 3.36 A 1.2% ethidium bromide-stained agarose gel showing the first strand cDNA synthesized from total RNA of testes of *P. monodon*. Lanes M and m = a 100 bp DNA ladder and λ-*Hin*d III, respectively. Lane 1-7 = the first strand cDNA from different individuals of *P. monodon*

Seven primer pairs were designed from nucleotide sequences of ESTs established from testes (*ubiquitin carboxyl-terminal hydrolase 14, ubiquitin carboxyl-terminal hydrolase 5*, *ubiquitin conjugating enzyme 2*, *PCTAIRE protein* (*Cdk17), kinase 2*, *dynein light intermediate chain*, *serine/threonine-protein kinase 23, proteasome alpha subunit*) of *P. monodon*. An additional primer pairs was designed

from an EST (*proteasome delta*) of hemocyte cDNA library. RT-PCR was carried out using an identical amplification condition for all primer pairs.

Expression patterns of *ubiquitin carboxyl-terminal hydrolase 14, ubiquitin carboxyl-terminal hydrolase 5*, *ubiquitin conjugating enzyme 2*, *PCTAIRE protein* (*Cdk17), kinase 2*, *dynein light intermediate chain*, *serine/threonine-protein kinase 23, proteasome alpha subunit* and *proteasome delta* were non-quantitatively examined by RT-PCR using cDNA from testes and ovaries of both juvenile and broodstock *P. monodon* (N = 3 for each group) as the template. Interestingly, all transcripts were more preferentially expressed in ovaries than testes of *P. monodon* (Figure 3.37-3.42).

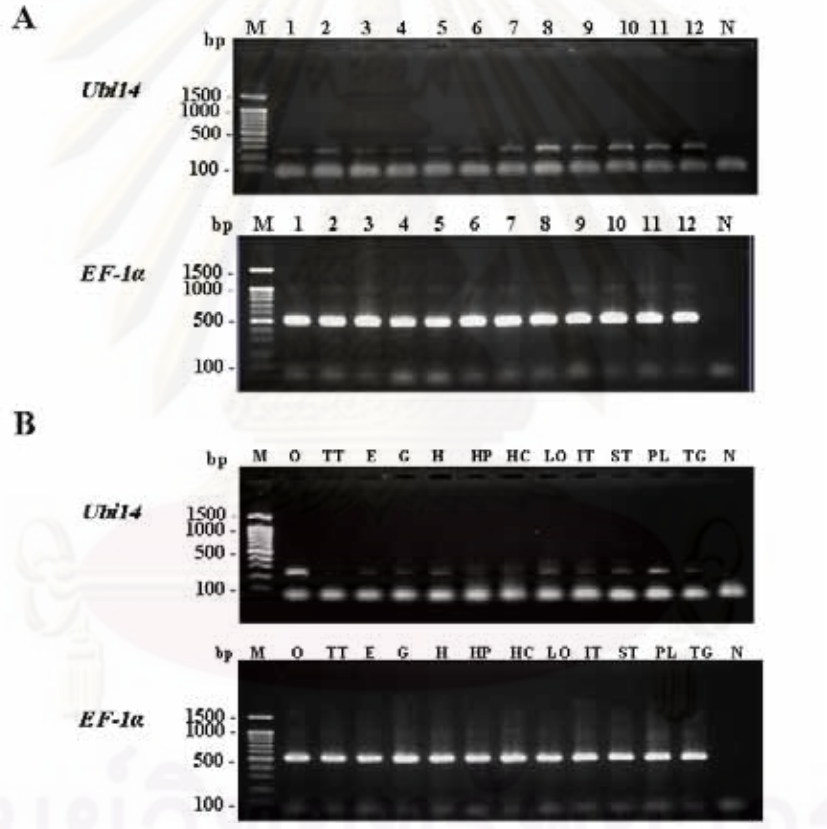


Figure 3.37 RT-PCR (A) and tissue distribution analysis (B) of *ubiquitin carboxyl-terminal hydrolase 14* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *ubiquitin carboxyl-terminal hydrolase 14* in different tissues was carried out using the cDNA template from ovaries (O), testes (TT), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (LO), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

Subsequently, tissue distribution analysis of these gene homologues was carried out in testes, heart, hemocytes, lymphoid organs, intestine, gills, pleopods, thoracic ganglion, stomach, eyestalk and hepatopancreas of a male broodstock and ovaries of a female broodstock of *P. monodon*.

Ubiquitin carboxyl-terminal hydrolase 14 was more abundantly expressed in ovaries and pleopods than other tissues (Figure 3.37). *Ubiquitin carboxyl-terminal hydrolase 5* was more abundantly expressed in ovaries, hemocytes and lymphoid organ than other tissues (Figure 3.38).

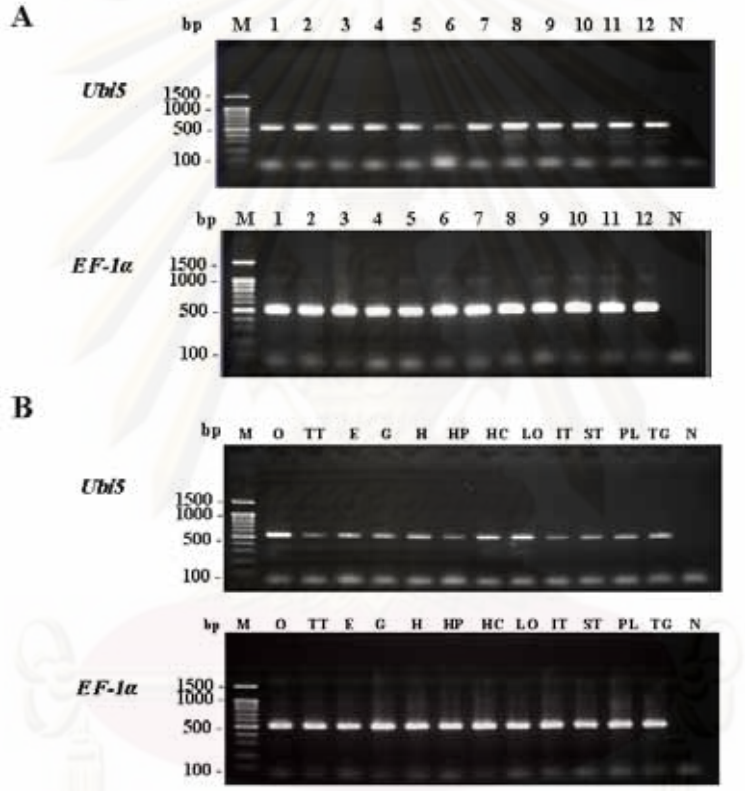


Figure 3.38 RT-PCR (A) and tissue distribution analysis (B) of *ubiquitin carboxyl-terminal hydrolase 5* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *ubiquitin carboxyl-terminal hydrolase 5* in different tissues was carried out using the cDNA template from ovaries (O), testes (TT), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (LO), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

Ubiquitin conjugating enzyme 2 was highly abundant expressed in ovaries. Moderately abundant expression was observed in heart, hemocytes, stomach and thoracic ganglion and low abundant expression was found in the remaining tissues (Figure 3.39).

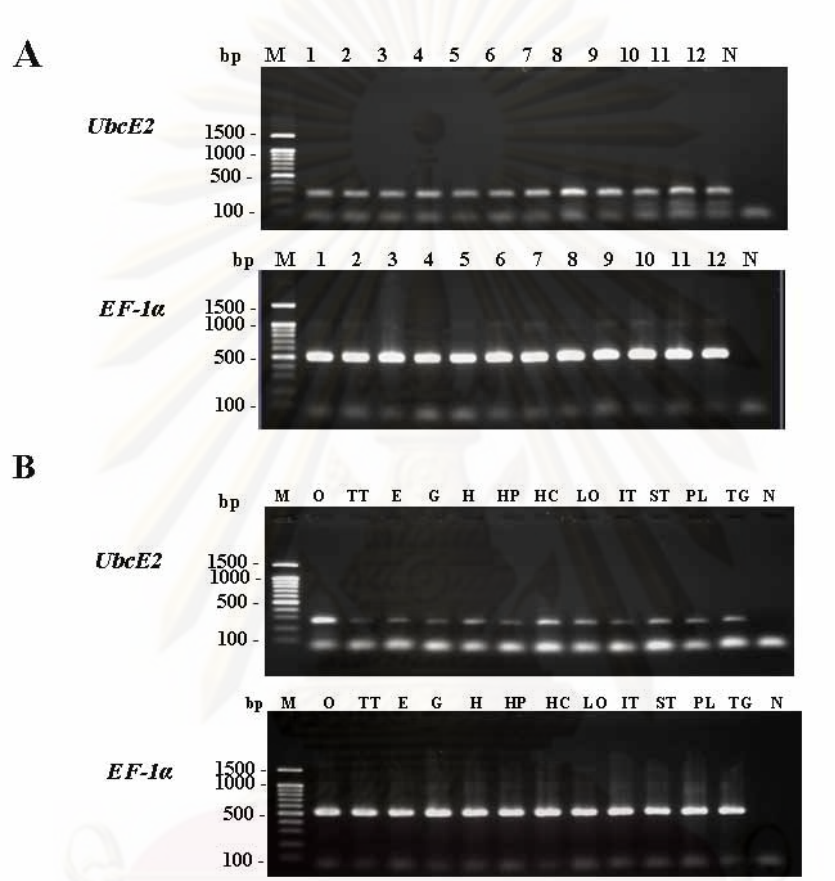


Figure 3.39 RT-PCR (A) and tissue distribution analysis (B) of *ubiquitin conjugating enzyme 2* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *ubiquitin conjugating enzyme 2* in different tissues was carried out using the cDNA template from ovaries (O), testes (TT), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (LO), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

In contrast, the expression of *proteasome alpha subunit* was quite low in most tissues except ovaries, heart, hemocytes and lymphoid organ (Figure 3.40).

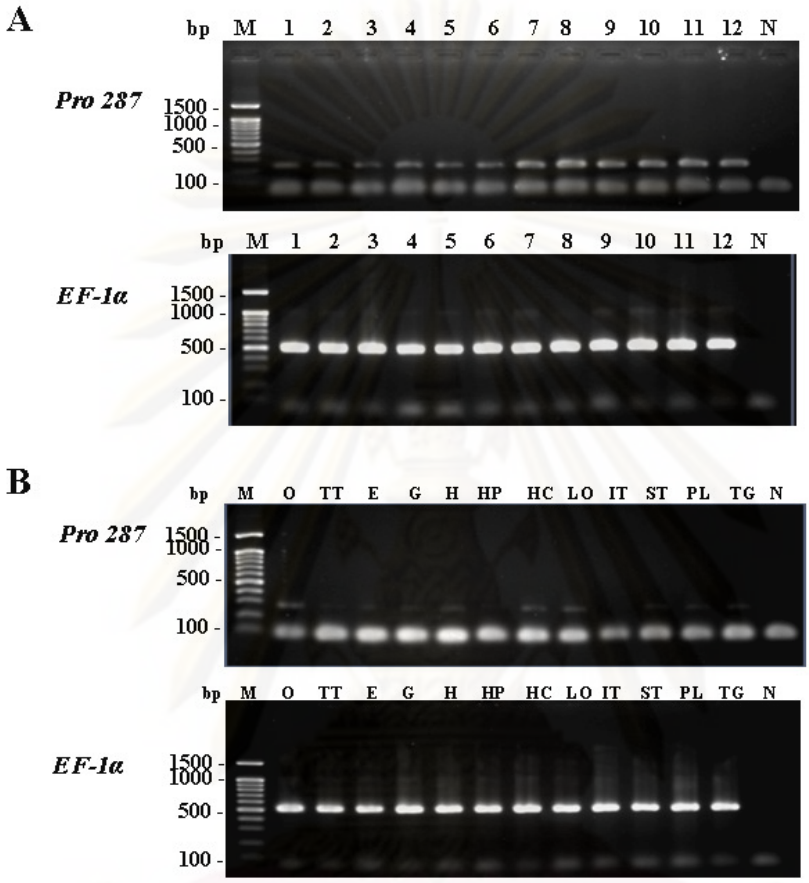


Figure 3.40 RT-PCR (A) and tissue distribution analysis (B) of *proteasome alpha subunit* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *proteasome alpha subunit* in different tissues was carried out using the cDNA template from ovaries (O), testes (TT), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (LO), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

Cdk17 (also called *PCTK2*) was more abundantly expressed in ovaries, hemocytes, lymphoid organ, pleopod and thoracic ganglion than testes, hepatopancreas and stomach. The expression of this transcript in eyestalk was rare (Figure 3.41).

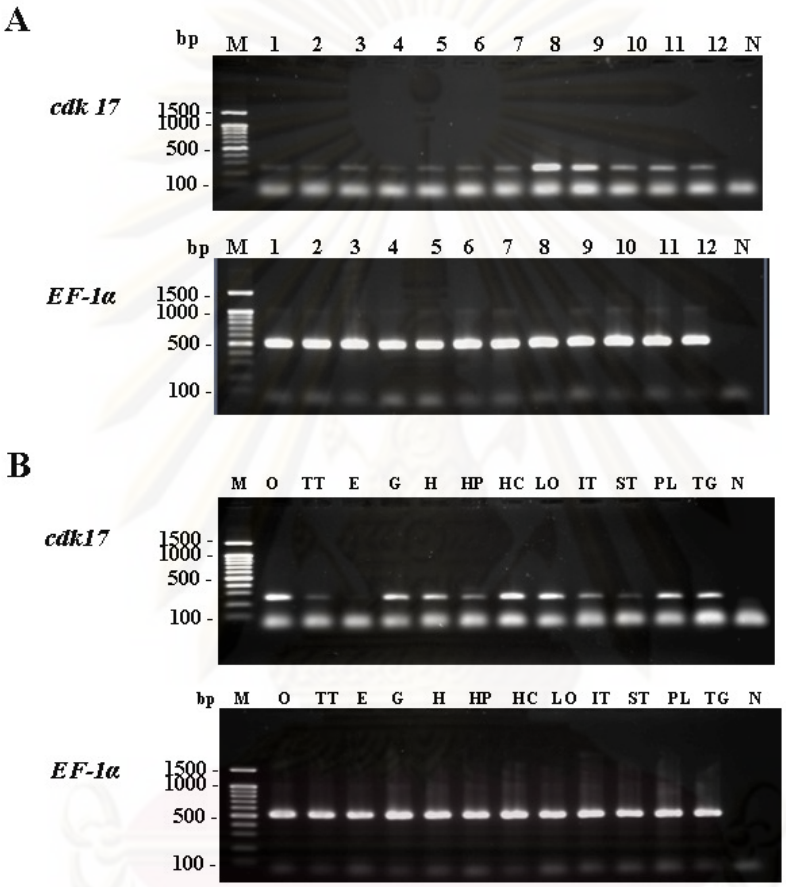


Figure 3.41 RT-PCR (A) and tissue distribution analysis (B) of *Cdk17* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *Cdk17* in different tissues was carried out using the cDNA template from ovaries (O), testes (TT), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (LO), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

Serine/threonine-protein kinase 23 was constitutively expressed in all examined tissues of *P. monodon* broodstock (Figure 3.42).

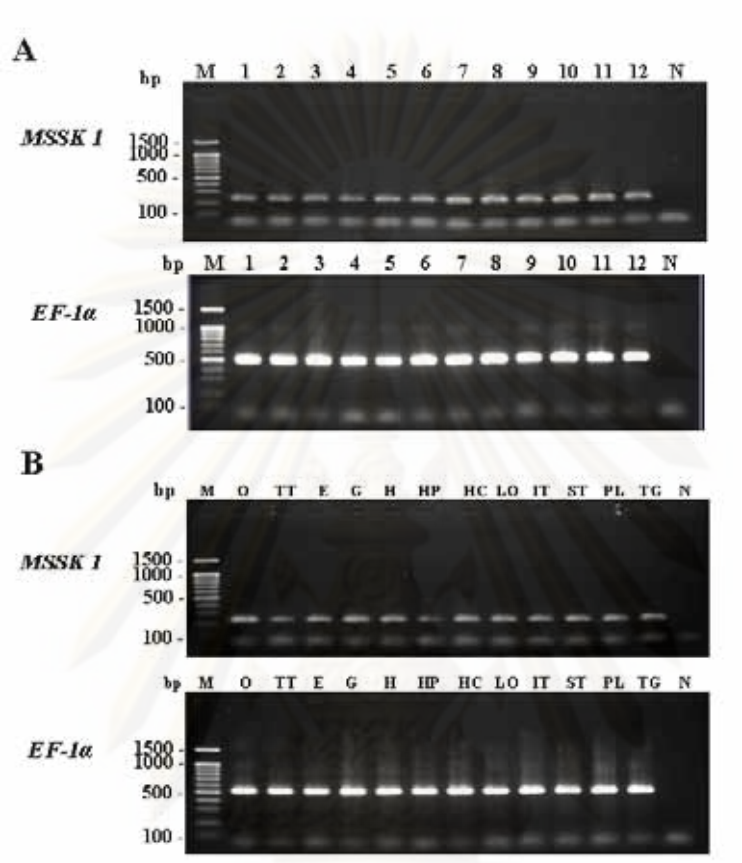


Figure 3.42 RT-PCR (A) and tissue distribution analysis (B) of *serine/threonine-protein kinase 23* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *proteasome alpha subunit* in different tissues was carried out using the cDNA template from ovaries (O), testes (TT), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (LO), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

Surprisingly, high abundant expression of *dynein light intermediate chain* was observed in ovaries. Extremely rare expression was observed in other tissues (Figure 3.43).

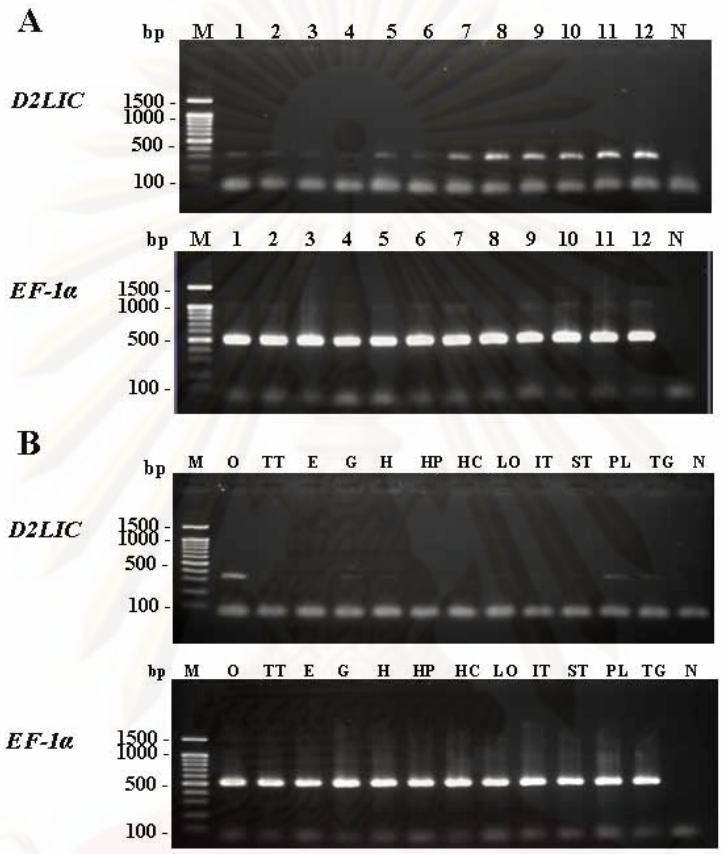


Figure 3.43 RT-PCR (A) and tissue distribution analysis (B) of *dynein light intermediate chain* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *dynein light intermediate chain* in different tissues was carried out using the cDNA template from testes (TT), ovaries (O), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (L), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

The highest expression level of *proteasome delta* was observed in ovaries. Comparably lower expression of this transcript was found in the remaining tissues except testes (Figure 3.44).

A summary for tissue expression analysis of all transcripts are illustrated by Table 3.7.

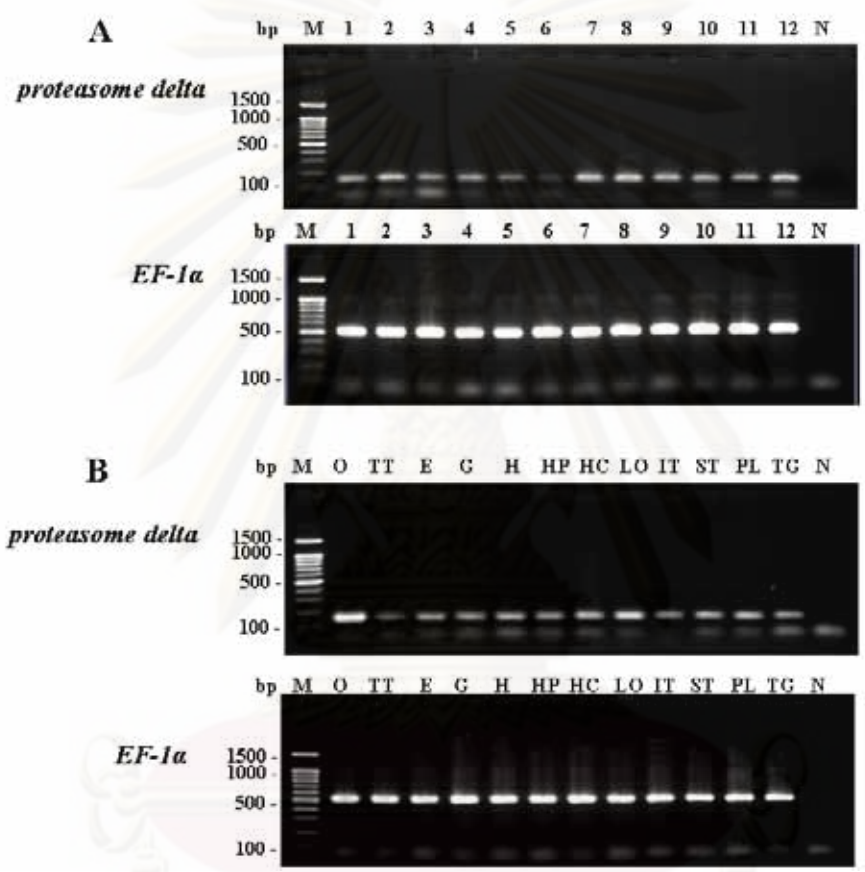


Figure 3.44 RT-PCR (A) and tissue distribution analysis (B) of *proteasome delta* using the first strand cDNA of testes of broodstock (lanes 1–3, A) and juveniles (lanes 4–6, A) and ovaries of broodstock (lanes 7–9, A) and juveniles (lanes 10–12, A) of *P. monodon*. Expression of *proteasome delta* in different tissues was carried out using the cDNA template from ovaries (O), testes (TT), eyestalks (E), gills (G), heart (H), hepatopancreas (HP), hemocytes (HC), lymphoid organs (L), intestine (IT), stomach (ST), pleopods (PL), and thoracic ganglion (TG). *EF-1α* was included as the positive control.

Table 3.7 Tissue expression of various functionally important genes in different tissues of *P. monodon* broodstock

Gene homologues	Amplicon size (bp)	ES	IT	ST	GL	HC	TG	LO	HP	HE	TT	PL	OV
1. *Ubiquitin carboxyl-terminal hydrolase 14*	240	+	+	+	+	+	+	+	+	+	+	++	++
2. *Ubiquitin carboxyl-terminal hydrolase 5*	525	+	+	+	+	++	+	++	+	+	+	+	++
3. *Ubiquitin conjugating enzyme 2*	262	+	+	++	+	++	++	+	+	++	+	+	+++
4. *Proteasome alpha subunit*	250	+	+	+	+	++	+	++	+	++	+	+	++
5. *Cdk17*	250	+	++	++	++	+++	+++	+++	++	++	++	+++	+++
6. *Serine/threonine-protein kinase 23* (*MSSK-1*)	229	++	++	++	++	++	++	++	++	++	++	++	++
7. *Dynein light intermediate chain*	324	+	+	+	+	+	+	+	+	+	+	+	+++
8. *Proteasome delta*	146	++	++	++	++	++	++	++	++	++	+	++	+++

+++ = high abundant expression, ++ = moderate abundant expression, + = low expression

ES = eyestalks, IT = intestine, ST = stomach, GL = gill, HC = hemocytes, TG = thoracic ganglion, LO = lymphoid organs, HP = hepatopancreas, HE = heart, TT = testes, PL = pleopods, OV = ovaries

3.5 Quantitative real-time PCR analysis of *serine/threonine-protein kinase 23*, *proteasome alpha subunit, 26S proteasome regulatory subunit S3*, *proteasome delta* and *ubiquitin carboxyl-terminal hydrolase 14* in testes of *P. monodon*

The expression levels of genes related to testicular development including *serine/threonine-protein kinase 23*, *26S proteasome regulatory subunit S3, proteasome alpha subunit*, *proteasome delta* and *ubiquitin carboxyl-terminal hydrolase 14* in testes of 6-month-old juvenile, domesticated broodstock: 10-month-old (DB10M-TT), 14-month-old (DB14M-TT) and 18-month-old (DB18M-TT) and wild broodstock were examined using quantitative real-time PCR analysis.

The standard curve of each gene was constructed from the 10-fold dilution covering 10^2 – 10^8 copy numbers of all genes except *EF-1α* where 10^3 – 10^8 copy numbers was used. The amplification efficiency of the target genes and the internal control, *EF-1α* are shown by Figure 3.45

Quantitative real-time PCR was carried out in duplicate using 250 ng of the first strand cDNA template for *ubiquitin carboxyl-terminal hydrolase 14*, 300 ng of the first strand cDNA template for *serine/threonine-protein kinase 23, proteasome alpha subunit*, *26S proteasome regulatory subunit S3* and *proteasome delta* and 10 ng of the first strand cDNA template for *EF-1α.*

3.5.1 *Serine/threonine-protein kinase 23*

Quantitative real-time PCR revealed that the expression levels of *serine/threonine-protein kinase 23* in testes of cultured 6-month-old juvenile and domesticated 10-month-old and 14-month-old broodstock were comparable ($P > 0.05$). Its expression level in 18-month-old shrimp seemed to be increased but it was not significant due to large standard deviation of the data set ($P > 0.05$). Interestingly, the express level of *serine/threonine-protein kinase 23* in testes of wild broodstock was not significantly different from that of juveniles and domesticated broodstock of *P. monodon* (Figure 3.46).

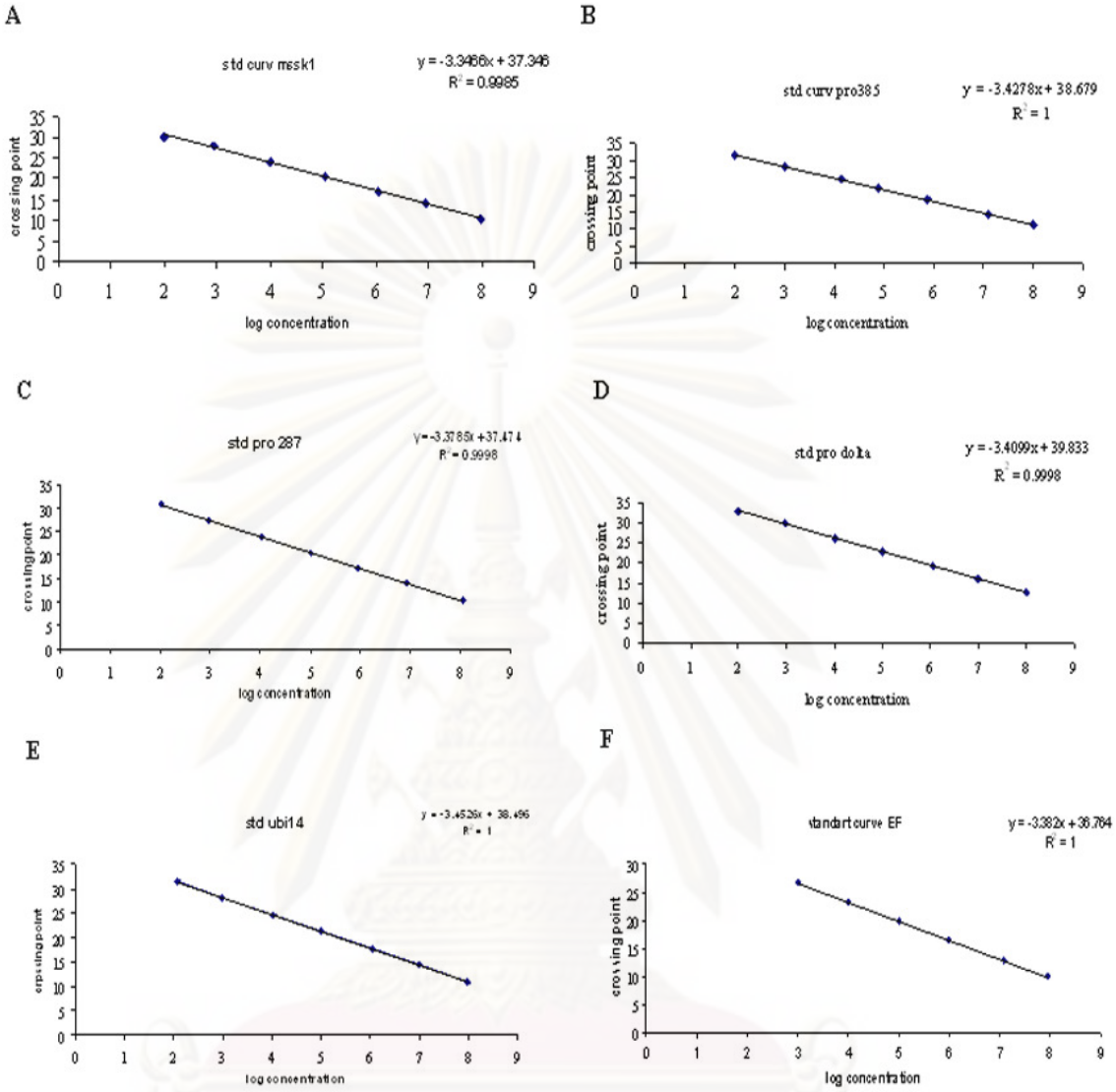
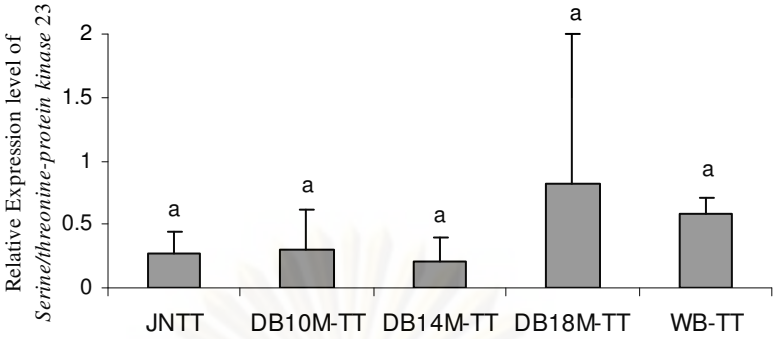


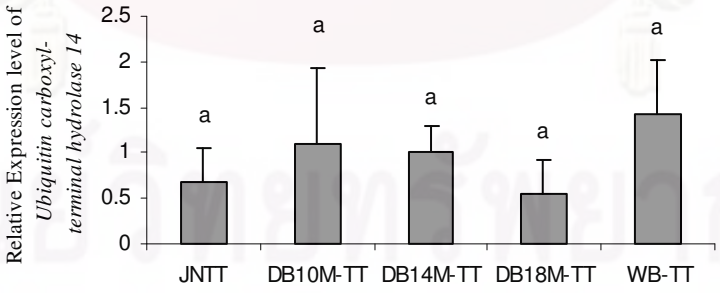
Figure 3.45 Standard curves of *serine/threonine-protein kinase 23* (A; R^2 = 0.999, efficiency = 1.950 and the equation Y = -3.347*log(X) + 37.346), 26S *proteasome regulatory subunit S3* (B; R^2 = 1.000, efficiency = 1.951 and the equation Y = -3.428 *log(X) + 38.679), *proteasome alpha subunit* (C; R^2 = 0.999, efficiency = 1.998 and the equation Y = -3.379*log(X) + 37.474), *proteasome delta* (D; R^2 = 0.999, efficiency = 1.952 and the equation Y = -3.4100*log(X) + 39.833), *ubiquitin carboxyl-terminal hydrolase 14* (E; R^2 = 1.000, efficiency = 1.951 and the equation Y = -3.453*log(X) + 38.496).



Figures 3.46 Histograms showing the relative expression levels of *serine/threonine-protein kinase 23* during testes development of 6-month-old male juveniles (JNTT), and 10-month-old (DB10M-TT), 14-month-old (DB14M-TT) and 18-month-old (DB18M-TT) domesticated male broodstock and wild (WB-TT) male broodstock of *P. monodon*.

3.5.2 *Ubiquitin carboxyl-terminal hydrolase 14*

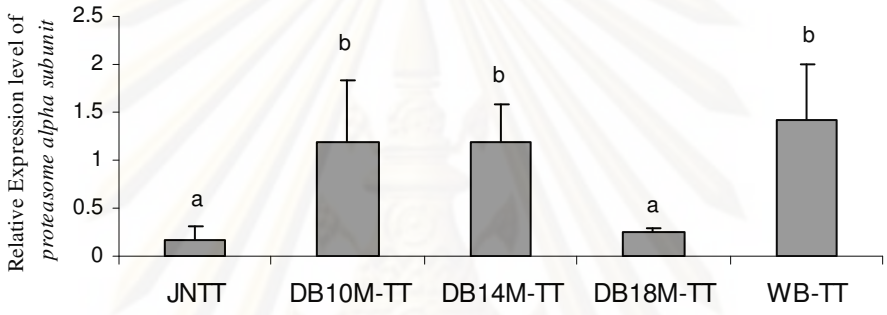
Quantitative real-time PCR revealed that the expression level of testicular *ubiquitin carboxyl-terminal hydrolase 14* seemed to be increased from 6-month-old juveniles in 10-month-old and 14-month-old broodstock and returned to the lowest level in 18-month-old broodstock. Nevertheless, these results were not statistically significant owing to the large standard deviation within each treatment group ($P > 0.05$). The expression level of *ubiquitin carboxyl-terminal hydrolase 14* in wild broodstock was not significantly different from that of juveniles and domesticated broodstock of *P. monodon* (Figure 3.47).



Figures 3.47 Histograms showing relative expression levels of *ubiquitin carboxyl-terminal hydrolase 14* during testes development of 6-month-old male juveniles (JNTT), and 10-month-old (DB10M-TT), 14-month-old (DB14M-TT) and 18-month-old (DB18M-TT) domesticated male broodstock and wild (WB-TT) male broodstock of *P. monodon*.

3.5.3 *Proteasome alpha subunit*

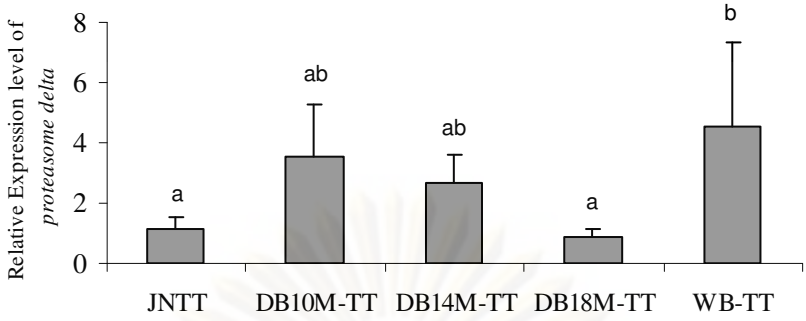
The expression level of *proteasome alpha subunit* in testes of 6-month-old juveniles was significantly lower than that of domesticated 10-month-old and 14-month-old broodstock ($P < 0.05$). The expression level of this gene was significantly decreased in 18-month-old broodstock ($P < 0.05$). The expression level of *proteasome alpha subunit* in testes of wild broodstock was not significantly different from that of domesticated 10-month-old and 14 month-old broodstock ($P > 0.05$) (Figure 3.48).



Figures 3.48 Histograms showing relative expression levels of *proteasome alpha subunit* during testes development of 6-month-old male juveniles (JNTT), and 10-month-old (DB10M-TT), 14-month-old (DB14M-TT) and 18-month-old (DB18M-TT) domesticated male broodstock and wild (WB-TT) male broodstock of *P. monodon*.

3.5.4 *Proteasome delta*

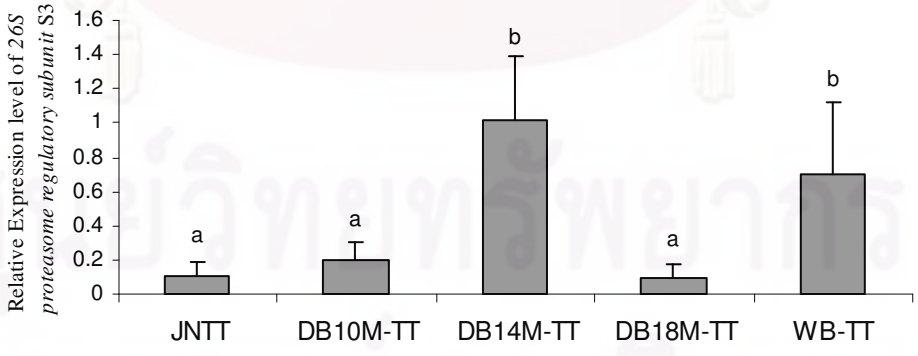
The expression levels of *proteasome delta* in testes of 6-month-old juveniles and 18-month-old broodstock were significantly lower than that of wild broodstock ($P < 0.05$). Nevertheless, its expression levels in 10-month-old and 14-month-old domesticated broodstock were not different from that in wild broodstock ($P > 0.05$) (Figure 3.49).



Figures 3.49 Histograms showing relative expression levels of *Proteasome delta* during during testes development of 6-month-old male juveniles (JNTT), and 10-month-old (DB10M-TT), 14-month-old (DB14M-TT) and 18-month-old (DB18M-TT) domesticated male broodstock and wild (WB-TT) male broodstock of *P. monodon*.

3.5.5 *26S proteasome regulatory subunit S3*

The expression levels of *26S proteasome regulatory subunit S3* in testes of 6-month-old juveniles and domesticated 10-month-old broodstock were not significantly different ($P > 0.05$). Nevertheless, its expression level was significantly increased in 14-months-old domesticated shrimp ($P < 0.05$) before returned to the basal level in 18-month-old broodstock ($P > 0.05$). The expression level of *26S proteasome regulatory subunit S3* in 14-month-old was not significantly different from that in wild male broodstock of *P. monodon* ($P > 0.05$) (Figure 3.50).



Figures 3.50 Histograms showing relative expression levels of *26S proteasome regulatory subunit S3* during testes development of 6-month-old male juveniles (JNTT), and 10-month-old (DB10M-TT), 14-month-old (DB14M-TT) and 18-month-old (DB18M-TT) domesticated male broodstock and wild (WB-TT) male broodstock of *P. monodon*.

CHAPTER IV

DISCUSSION

Proteomic studies of proteins in testes of *P. monodon*

Isolation and characterization of reproduction-related genes in testes of *P. monodon* have been reported based on EST analysis (Leelatanawit et al., 2004; 2008 and 2009). Nevertheless, identification of the gene products at the protein levels which provides more direct information of molecule functions on testicular development of this species has not been reported.

Bulau et al. (2004) characterized neuropeptides from the X-Organ-sinus gland neurosecretory system of the crayfish, *Orconectes limosus* using a nanoflow liquid chromatography system coupled to quadrupole-time-of-flight tandem mass spectrometry (nanoLC-QTOF MS/MS). The existence and structural identity of four crustacean hyperglycemic hormone precursor-related peptide variants and two new genetic variants of the pigment-dispersing hormone, not detected by conventional chromatographic systems, molecular cloning, or immunochemical methods before, was revealed.

In decapod crustaceans, the regulation of the molting cycle involves 2 endocrine organs: the X-organ/sinus gland (XO/SG) complex located in the eyestalk ganglia and the Y-organ (YO) located in the cephalothorax. Molt-inhibiting hormone (MIH) and crustacean hyperglycemic hormone (CHH) are produced in the XO/SG complex and inhibit ecdysteroidogenesis in the YO. Thus, YO activation is induced by eyestalk ablation (EA), which removes the primary source of MIH and CHH. Lee and Mykles (2006) used proteomics to identify potential components of signal transduction pathways ("targeted" or cell-map proteomics) as well as assess the magnitude of protein changes in response to activation ("global" or expression proteomics) in the tropical land crab (*Gecarcinus lateralis*). Total proteins in YOs from intact and ES-ablated animals were separated by 2-DE and expression profiles were assessed by image analysis and gene clustering software. EA caused a >3-fold increase in the levels of 170 proteins and >3-fold decrease in the levels of 89 proteins;

a total of 543 proteins were quantified in total YO extracts. EA induced significant changes in the levels of 3 groups of proteins eluting from a phosphoprotein column and detected with phosphoprotein staining of 2-DE gels; ~17 kDa and ~150 kDa phosphoproteins increased in activated YOs, while ~12 kDa phosphoproteins decreased. A ~150 kDa phosphoprotein, which was isolated only from activated YO, was identified as NO synthase by western blotting and mass spectrometry of trypsin-digested peptides. The data illustrated that phosphorylation of NO synthase is associated with activation of the YO.

In an effort to better understand testicular development, typical 2-DE gel electrophoresis and mass spectrometry based on LC-MS/MS was used for isolation and characterization of protein profiles in testes (excluding the sperm sac) of wild (GSI =1.08 ± 0.18% and sperm sac/testis = 0.26 ± 0.06 N = 3) and domesticated *P. monodon* (GSI = 0.37 ± 0.05%, sperm sac/testis = 0.22 ± 0.01, N = 3 and GSI = 0.31 ± 0.05%, sperm sac/testis = 0.52 ± 0.02, N = 3). The broad pH gradient (pH 3-10) revealed that a relatively large number of electrophoresed protein spots were found. Almost all of the expressed testicular proteins were acidic proteins and much lower numbers of basic proteins were observed in testis of *P. monodon***.**

A total of 640 protein spots were characterized including 394 spots from wild broodstock (group A), 120 spots from domesticated broodstock group B and 126 sports from domesticated broodstock group C. Novel proteins predominated and 254 (55.31%) spots were found. A total of 208 spots (32.50%) significantly matched sequences in the database and considered as known proteins. In addition, unnamed (15 spots, 2.34%) and hypothetical (32 spots, 5.0%) proteins were found. Several reproduction-related proteins were identified for example, heat shock protein 90 (Hsp90), progesterone receptor-related protein p23, farnesoic-O-methyltransferase (FAMeT), cyclophilin A, NADP-dependent leukotriene B4 12-hydroxydehydrogenase (LTB4DH, receptor for activated protein kinase C (RACK), 14-3-3 like protein and several members of ubiquitin-proteosome pathways (e.g. proteosome delta, proteasome subunit alpha type).

The actions of progesterone are mediated through binding to the nuclear progesterone receptor, a member of the steroid/thyroid hormone receptor superfamily, as the classical pathway (Rao et al., 1974; Evans, 1988). Progesterone receptor-related

protein p23 (p23) was first characterized and named as an essential component of the Hsp90 molecular chaperone complex with the progesterone receptor (Johnson and Toft, 1994). p23 binds the ATP-bound form of Hsp90 and blocks its ATPase activity, thereby stabilizing that state and thus client protein binding (Felts and Toft, 2003).

Methyl farnesoate (MF) is the crustacean homolog of the insect juvenile hormone (JH-III) and is believed to regulate growth and reproduction in crustaceans (Huberman, 2000). MF is synthesized in mandibular organ (MO) from farnesoic acid (FA) by the action of farnesoic acid O-methyltransferase (FAMeT) in the presence of *S*-adenosyl methionine (Nagaraju, 2007). It has been reported that MF maintain juvenile morphology and, therefore, inhibits gonadal development in juveniles but enhances reproductive maturation in adults (Borst and Laufer, 1990; Nagaraju et al., 2003).

$NADP^+$-dependent leukotriene B4 12-hydroxydehydrogenase (LTB4DH) is the key enzymes responsible for biological inactivation of prostaglandins and related eicosanoids.

Cyclophilins are small proteins that bind Cyclosporin A (CsA) and catalyze protein folding (Lage et al., 1987). Cyclophilins are characterized by a conserved CBD that is required for both CsA-binding and protein-folding activities (Page et al., 1996). Recently, a diverse cyclophilin, mog 1 was isolated and functionally characterized. Binding of mog 1 to MEP-1 is essential for germline sex determination in *Caenorhabditis elegans* (Belfiore *et al*., 2004).

The full length cDNA of *progesterone receptor-related protein p23* (Preechaphol, 2009), *LTB4DH* (Praserlux, 2006), *RACK* (Buaklin, 2005) and *FAMeT* (A. Buaklin, unpublished data) in ovaries and *cyclophilin A* (Leelatanawit, 2008) in testes of *P. monodon* were successfully identified and characterized. The expression levels of these genes during ovarian/testicular development of *P. monodon* were examined. In addition, the functional importance of these proteins on reproductive maturation of *P. monodon* should also be carried out.

Silver staining is a sensitive technique for detection proteins of the polyacrylamide gels. However, silver staining leads to a non-stoichiometric binding of silver ions to proteins. After reduction, these complexes become visible as black to

brownish bands. However, some proteins are hardly stained by silver ions. Therefore, quantity of stained proteins is not proportionally indicated from intensity of the protein spots. As a result, the intensity of proteins identified by 2-DE electrophoresis in this study was not examined.

The use of 2-DE for proteomic analysis is tedious and time consuming. In addition, it is difficult (or not possible) to identify proteins with very low and high molecular weight or those exhibiting very low or high p*I* simultaneously. In this thesis, one-dimensional gel electrophoresis (SDS-PAGE) was also used and the electrophoresed proteins were further characterized by LC-MS/MS. In this case, the protein staining method does not interfere the ability to compare whether the examined proteins were differentially expressed or not as the intensity of each protein in different specimens was evaluated from LC-MS/MS spectrum results.

The size-fractionated proteins patterns of wild male *P. monodon* broodstock could be divided to 2 groups and these samples (N = 3 for each group) and were analyzed separately. In addition, size-fractionated proteins from testes of 14-month-old (N = 3) and 18-moth-old (N = 3) males were also examined. The intensity of the protein spectrum from testes of wild broodsotck group A was used to normalize that of other sample groups. Based on the fact that a few thousands of different proteins were identified for each molecular weight range, approximately 50 proteins that showed large differential (up-regulation and down-regulation based on the evaluation from mass spectrometry results) expression profiles among sample groups were annotated.

For a proteomic approach based on 1-DE gel electrophoresis, 345 differentially expressed proteins were identified. Of which, 223 (64.64%) proteins significantly matched known proteins in the database and 122 (35.36%) proteins did not matched any proteins and were considered as unknown proteins.

Interestingly, 1 (0.29%; GK24443) protein was found only in group A. In addition, 18 (5.22%; e.g. p97/VCP-binding protein p135, lipoxygenase homology domains 1, dipeptidyl-peptidase and SEParase family member, sep-1) were found in both groups of wild broodstock but not in domesticated broodstock while 231 (66.96%; e.g. vasa-like protein, Ran GTPase activating protein 1, seven membrane

helix receptor, nuclear receptor subfamily 3, zinc finger protein 184 retinoblastoma binding protein) proteins were commonly found in all groups of samples.

The *vasa-like protein* encodes an ATP-dependent RNA helicase belonging to the DEAD-box family that, in many organisms, is specifically expressed in germline cells throughout the life cycle. Recently, Aflalo *et al.* (2006) characterized the full length cDNA of *vasa-like protein* in *L. vannamei*. This gene was only expressed in shrimp gonads. The *vasa-like protein* transcript is localized to the cytoplasm of oocytes throughout oogenesis. The identification of this protein in testes of *P. monodon* allows functional analysis of this protein and enhances the understanding of developmental and reproductive processes in the germline of this species.

The expression profiles of proteins found only in wild broodstocks may be used as biomarkers for reduced reproductive maturation of *P. monodon* in captivity. In addition, negative or positive effects of the key proteins on the progression of testicular development may also inferred from up- or down-regulated proteins compared between wild and domesticated *P. monodon*. Importantly, the preliminary data on differential expression profiles of key testicular proteins of *P. monodon* should be further confirmed by Western blot analysis or ELISA.

Proteomic analysis based on 1-DE (SDS-PAGE) is more convenient and cost-effective than that based on the conventional 2-DE approach. In addition, differentially expressed proteins were effectively determined disregarding the staining method. Typically, extensive proteomic analysis is prohibited by the cost of mass spectrometry for each protein spot. The new technique described in this study allow simple and possible opportunity to apply proteomics for determining various aspects related with reproductive maturation in male *P. monodon* for which the information is not available at present.

In this study, a large number of proteins including sex-related in testes of *P. monodon* were identified. The expression profiles of proteins specifically expressed or those preferentially expressed in testes of *P. monodon* implied that these proteins may have contributed testicular development in *P. monodon*. Functionally analysis of proteins involving testicular development can be further carried out for better understanding of the reproductive maturation of male *P. monodon*.

Isolation of the full length cDNA and expression analysis of functionally important genes in testes of *P. monodon*

Ubiquitin-dependent proteolysis mediates selective destruction of some important proteins, such as various cell cycle regulators, transcription factors and tumor suppressors. The concentrations of key proteins in diverse regulatory pathways are controlled by posttranslational ubiquitination and degradation by the 26S proteasome (Deng et al., 2007). Therefore, alterations in this proteolytic system should be associated with a variety of pathways necessary for testicular development of *P. monodon*.

Ubiquitin specific proteases (USPs) belong to a complex family of deubiquitinating enzymes that specifically cleave ubiquitin conjugates on a great variety of substrates, thereby, USPs regulate the production and recycling of ubiquitin and are critically involved in the control of cell growth, differentiation, and apoptosis of organisms (Ovaa et al., 2004; Rolen et et al., 2006).

Cyclin-dependent kinases (Cdks) are protein kinases involved in critical cellular processes, such as cell cycle or transcription, whose activity requires association with specific cyclin subunits.

In this study, the full length cDNA of *ubiquitin specific peptidase 14* (ORF of 1524 bp corresponded to a polypeptide of 507 amino acids), *ubiquitin carboxyl-terminal hydrolase 5* (ORF of 2442 bp corresponded to a polypeptide of 813 amino acids), *Cdk17* (ORF of 1470 bp corresponded to a polypeptide of 489 amino acids) and *proteasome alpha subunit* (ORF of 765 bp corresponded to a polypeptide of 254 amino acids) of *P. monodon* was successfully identified and reported for the first time in this species.

Tissue distribution and expression levels of functionally important genes in testes of wild and domesticated *P. monodon*

One difficulty in identifying compounds that stimulate crustacean reproduction is the lack of adequate biological markers for reproductive maturation particularly, in *P. monodon*.

Gene expression and tissue distribution analysis are important and provide the basic information to set up the priority for further analysis of functional genes. A particular gene may express in several tissues and it may possess a different function in different tissues.

In the giant freshwater prawn (*Macrobrachium rosenbergii*), a suppression subtractive hybridization (SSH) male reproductive tract library was constructed to identify male-specific genes that could be involved in male development. A novel Mar-Mrr (*M. rosenbergii* male reproduction-related gene, 683 bp in length with an ORF of 333 bp) and the Kazal-type peptidase inhibitor (KPI, 736 bp, ORF of 405 bp) transcripts were identified and these genes were only expressed in the male reproductive tract of *M. rosenbergii* (Cao et al., 2006, 2007).

Leelatanawit (2008) examined expression patterns of 59 gene homologues in testes and ovaries of juvenile and broodstock *P. monodon* ($N = 4$ for each group) by non-quantitative RT-PCR. *PmTST1* was only expressed in testes ($N = 8$) but not ovaries ($N = 8$) whereas *multiple inositol polyphosphate phosphatase 2* (*MIPP2*) and *HSP70-2* exhibited a trend of preferential expression in testes of *P. monodon*. Thirty-six genes showed a trend of greater expression levels in ovaries than testes. In addition, semi-quantitative RT-PCR and quantitative real-time PCR were carried out to examine expression levels of 12 gene homologues in different groups of shrimp. Testis-specific expression of *PmTST1* was confirmed. *Cyclophilin A* (*CYA*) and *thyroid hormone-associated protein, 240 kDa* (*Trap240*) were more abundantly expressed in ovaries than testes ($P < 0.05$). *Dmc1*, *saposin*, *spermatogonial stem-cell renewal factor*, *MIPP* and *HSP70-2* were preferentially expressed in testes to ovaries ($P < 0.05$). Expression levels of *SUMO-1*, *Tra-2* and *prohibitin2* in ovaries and testes of *P. monodon* were not significantly different ($P > 0.05$). *PMTST1* was up-regulated but that of the remaining genes in testes of *P. monodon* broodstock was down-regulated after shrimp were molted ($P < 0.05$). Significant reduction of *SUMO-1*, *Dmc1*, and *spermatogonial stem-cell renewal factor* and increment of *prohibitin2* transcripts in domesticated broodstock ($P < 0.05$) suggested that these reproductively related genes may be used as biomarkers to evaluate reduced degrees of the reproductive maturation in domesticated *P. monodon*.

Tissues distribution analysis of *ubiquitin carboxyl-terminal hydrolase 14, ubiquitin carboxyl-terminal hydrolase 5*, *ubiquitin conjugating enzyme 2*, *cdk17*, *dynein light intermediate chain*, *serine/threonine-protein kinase 23, proteasome alpha subunit* and *proteasome delta* were examined in various tissues of a male broodstock and ovaries of a female broodstock. These genes were not specifically expressed in gonads of shrimp but widely expressed in various tissues. This suggested that their gene products may play multifunctional properties in different tissues of *P. monodon*.

The transcriptional levels of preferentially expressed genes in testes could be used as the responsive indicators for reproductive maturation of *P. monodon*. Although *serine/threonine-protein kinase 23* and *ubiquitin carboxyl-terminal hydrolase 14* did not reveal differential expression profiles in different groups of male *P. monodon* ($P > 0.05$), the expression levels of *proteasome alpha subunit* and *proteasome delta* in testes of 10- and 14-month-old shrimp was not significantly different from those of wild broodstock ($P < 0.05$) but significantly different from those of 6-month-old juveniles and 18-month-old broodstock ($P > 0.05$). As a result, the expression profiles of testicular *proteasome alpha subunit* and *proteasome delta* indicated that domesticated male shrimp possibly reached the maturation period at about 10- 14 months of the cultivation period.

In addition, the expression levels of *26S proteasome regulatory subunit S3* in testes of domesticated 14-month-old broodstock and wild broodstock were not different ($P > 0.05$). In contrast, the expression level of this transcript in 6-month-old juveniles and domesticated 10- and 18-month-old broodstock were significantly lower than that of 14-month-old shrimp ($P < 0.05$). The expression profiles of of *26S proteasome regulatory subunit S3* further indicated that domesticated male shrimp possibly reached the maximal maturation at 14-month-old and maturation of domesticated males may be reduced afterwards.

In female *P. monodon*, different developmental stages of ovaries could be simply inferred from the GSI values of female shrimp (e.g. < 1.5, >2 - 4, > 4-6 and > 6% for stages I – IV ovaries, respectively). Practically, ovarian developmental stages of female *P. monodon* could be examined externally by farmers. However, this approach could not be applied to evaluate the developmental stages of testes in male

P. monodon. Therefore, biomarkers for evaluation of degrees of testicular maturation of *P. monodon* are needed.

The GSI values of wild broodstock were greater than those of domesticated broodstock even though their body weights were comparable implying a possible reduction of the maturation potential in domesticated shrimp. The expression profiles of genes preferentially expressed in testes of *P. monodon* illustrated in this study suggested that these genes may have contributed testicular development in *P. monodon*. Practically, biomarkers to indicate male maturation should be developed based on the non-lethal sampling method. This could be done be by further analysis on the expression profiles of *proteasome alpha subunit*, *proteasome delta* and *26S proteasome regulatory subunit S3*, for example, in hemocytes of male *P. monodon*.

In this study, genes/proteins expressed in testes of *P. monodon* were identified and characterized. The expression profiles of several reproduction-related transcripts were examined. Molecular mechanisms of genes and proteins controlling testicular maturation should be further carried out for better understanding the reproductive maturation of *P. monodon* in captivity.

CHAPTER V

CONCLUSION

1. Proteomic analysis based on 2-DE was carried out to identify reproduction-related proteins in testes of wild and domesticated 14-month-old broodstock of *P. monodon*. A total of 640 protein spots were characterized by nanoLC-MS/MS. Several reproduction-related proteins such as FAMeT, p23, RACK, 14-3-3-like protein and LTB4DH were identified.

2. Proteomic analysis based on 1-DE of proteins profiles in testes of wild (groups A and B), domesticated 14-month-old (group C) and 18-month-old (group D) broodstock of *P. monodon* was also carried out. In total, 345 differentially expressed proteins were identified. Of these, 1 (0.29%) and 18 (5.22%) proteins were found in only group A and both groups of wild broodstock. Several reproduction-related proteins such as vasa-like protein, Ran GTPase activating protein 1 and seven transmembrane helix receptor were identified.

3. The full length cDNA of *ubiquitin specific peptidase 14* (ORF of 1524 bp corresponded to a polypeptide of 507 amino acids), *ubiquitin carboxyl-terminal hydrolase 5* (ORF of 2442 bp corresponded to a polypeptide of 813 amino acids), *Cdk17* (ORF of 1470 bp corresponded to a polypeptide of 489 amino acids) and *proteasome alpha subunit* (ORF of 765 bp corresponded to a polypeptide of 254 amino acids) of *P. monodon* was successfully identified.

4. Tissue distribution analysis indicated that *ubiquitin specific peptidase 14*, *ubiquitin carboxyl-terminal hydrolase 5*, *Cdk17* and *proteasome alpha subunit* were constitutively expressed in all examined tissues of *P. monodon* broodstock.

5. The expression levels of *serine/threonine-protein kinase 23* and *ubiquitin carboxyl-terminal hydrolase 14* in testes of juveniles and domesticated and wild broodstock of male *P. monodon* were not significantly different ($P > 0.05$).

6. The expression levels of *proteasome alpha subunit* and *proteasome delta* in testes of 10- and 14-month-old shrimp was not significantly different from those of

wild broodstock ($P < 0.05$) but significantly greater than those of 6-month-old juveniles and 18-month-old broodstock ($P > 0.05$).

7. The expression levels of *26S proteasome regulatory subunit S3* in testes of domesticated 14-month-old broodstock and wild broodtock were not different ($P > 0.05$) but its expression level in 6-month-old juveniles and domesticated 10- and 18-month-old broodstock were significantly lower than that of 14-month-old shrimp ($P < 0.05$).

8. The information on proteins expressed in testes is useful for further studies on testicular development and spermatogenesis of *P. monodon*. The expression profiles of *proteasome alpha subunit*, *proteasome delta* and *26S proteasome regulatory subunit S3* illustrated that domesticated male *P. monodon* possibly reached the initial maturation period at 10 months, attained the maximal maturation peak at 14 months and reduced the reproductive maturation at 18 months of cultivation. The basic knowledge obtained could be applied for selection of the appropriate age of domesticated male broodstock used in the breeding programs of *P. monodon*.

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APPENDICES

APPENDIX A

Table. A1 Relative expression level data of *Serine/threonine-protein kinase 23* in testes of male broodstock *P. monodon* using real-time PCR.

Sample Group		concentration		Ratio of gene/*EF-1α*	Average	STD
		Serine/threonine-protein kinase 23	*EF-1α*			
1	jntt2	394.3595898	6005.282084	0.065668787		
	jntt3	497.3546758	1231.207252	0.403956909		
	jntt7	943.545239	2820.494462	0.334531853	.268053	.1786738
2	BU10MTT2	5507.596989	60896.86202	0.090441392		
	BU10MTT12	10177.88897	42378.31668	0.240167373		
	BU10MTT13	19410.70135	25780.72294	0.752915323		
	BU10MTT20	2128.033639	21269.86024	0.100049253	.295893	.3122712
3	BU14MTT17	6374.203492	28310.75869	0.225151278		
	BU14MTT22	18592.31478	47416.70829	0.392104713		
	BU14MTT37	831.1700986	71706.55215	0.011591271	.209616	.1907318
4	BU18MTT5	14992.46395	5161.119782	2.904885875		
	BU18MTT13	1487.174812	3773.373071	0.39412345		
	BU18MTT14	1175.187589	2432.283495	0.483162259		
	BU18MTT20	1333.993983	9508.663006	0.140292487		
	BU18MTT24	3459.299031	24166.02409	0.143147214	.813122	1.1791390
5	BFNTT1	3757.406616	6213.231903	0.604742697		
	BFNTT4	6050.986546	12295.31894	0.49213742		
	BFNTT8	8836.271954	12699.44504	0.69579985		
	BFNTT9	8338.246426	11823.28136	0.705239617		
	BFNTT10	10944.55895	26089.7276	0.419496866	.583483	.1256256

Table.A2 Relative expression level data *Ubiquitin carboxyl-terminal hydrolase 14* in testes of male broodstock *P. monodon* using real-time PCR.

Sample Group		concentration		Ratio of gene/*EF-1α*	Average	STD
		Ubiquitin carboxyl-terminal hydrolase 14	*EF-1α*			
1	jntt2	2509.140795	6005.282084	0.417822304		
	jntt3	1357.411673	1231.207252	1.102504611		
	jntt7	1375.669072	2820.494462	0.487740391	.669356	.3767434
2	BU10MTT2	26933.57987	60896.86202	0.442281901		
	BU10MTT12	51606.21761	42378.31668	1.21775053		
	BU10MTT13	57801.65647	25780.72294	2.24204948		
	BU10MTT20	9552.009687	21269.86024	0.449086622	1.087792	.8512398
3	BU14MTT17	26226.33371	28310.75869	0.926373397		
	BU14MTT22	62823.64836	47416.70829	1.324926395		
	BU14MTT37	55534.39908	71706.55215	0.774467568	1.008589	.2842900
4	BU18MTT5	2186.776021	5161.119782	0.423701854		
	BU18MTT13	429.1459534	3773.373071	0.113730062		
	BU18MTT14	2618.569974	2432.283495	1.076589131		
	BU18MTT20	6887.118208	9508.663006	0.724299326		
	BU18MTT24	9774.062811	24166.02409	0.404454733	.548555	.3657636
5	BFNTT1	5275.222229	6213.231903	0.849030313		
	BFNTT4	17931.06059	12295.31894	1.458364819		
	BFNTT8	22570.07941	12699.44504	1.777249269		
	BFNTT9	25822.66584	11823.28136	2.184052383		
	BFNTT10	21550.21685	26089.7276	0.826003904	1.418940	.5898532

Table. A3 Relative expression level data *Proteasome delta* in testes of male broodstock *P. monodon* using real-time PCR**.**

Sample Group		concentration		Ratio of gene/*EF-1α*	Average	STD
		Proteasome delta	*EF-1α*			
1	jntt2	5546.592259	6005.282084	0.923618938		
	jntt3	1064.71583	1231.207252	0.864773846		
	jntt7	4430.280545	2820.494462	1.570746053	1.119713	.3917127
2	BU10MTT2	214406.607	60896.86202	3.520815357		
	BU10MTT12	49037.22563	42378.31668	1.157130096		
	BU10MTT13	124223.9651	25780.72294	4.818482606		
	BU10MTT20	100092.2424	21269.86024	4.705825111	3.550563	1.7001624
3	BU14MTT17	45966.21575	28310.75869	1.623630658		
	BU14MTT22	161808.3007	47416.70829	3.412474348		
	BU14MTT37	208713.526	71706.55215	2.910661853	2.648922	.9226977
4	BU18MTT5	3689.456328	5161.119782	0.714855784		
	BU18MTT13	3349.219659	3773.373071	0.887593036		
	BU18MTT14	3111.66778	2432.283495	1.279319531		
	BU18MTT20	9036.154146	9508.663006	0.95030754		
	BU18MTT24	13500.13785	24166.02409	0.558641248	.878143	.2715620
5	BFNTT1	17951.61123	6213.231903	2.889254981		
	BFNTT4	71916.5927	12295.31894	5.84910347		
	BFNTT8	54255.89932	12699.44504	4.272304747		
	BFNTT9	29163.6412	26089.7276	1.117820839		
	BFNTT10	100116.9448	11823.28136	8.467779942	4.519253	2.8127827

Table. A4 Relative expression level data of *proteasome alpha subunit* in testes of male broodstock *P. monodon* using real-time PCR.

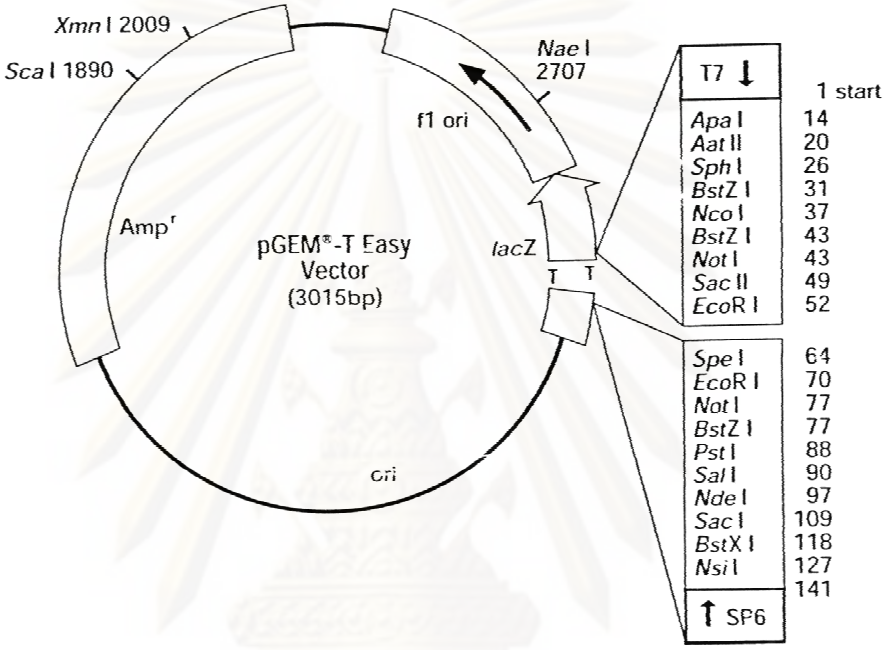
Sample Group		concentration		Ratio of gene/*EF-1α*	Average	STD
		proteasome alpha subunit	*EF-1α*			
1	jntt2	382.7177617	6005.282084	0.063730189		
	jntt3	394.3267656	1231.207252	0.320276513		
	jntt7	413.2049189	2820.494462	0.146500879	.176836	.1309357
2	BU10MTT2	73991.42358	60896.86202	1.215028511		
	BU10MTT12	11819.27783	42378.31668	0.278899181		
	BU10MTT13	43746.58008	25780.72294	1.696871735		
	BU10MTT20	33325.37643	21269.86024	1.566788688	1.189397	.6402131
3	BU14MTT17	23695.61336	28310.75869	0.836982634		
	BU14MTT22	76086.41454	47416.70829	1.604632993		
	BU14MTT37	81062.39797	71706.55215	1.130474072	1.190697	.3873523
4	BU18MTT5	1004.071374	5161.119782	0.194545257		
	BU18MTT13	994.4752206	3773.373071	0.263550728		
	BU18MTT14	755.9797641	2432.283495	0.310810712		
	BU18MTT20	1786.303497	9508.663006	0.187860638		
	BU18MTT24	5946.522927	24166.02409	0.246069561	.240567	.0509624
5	BFNTT1	4420.252382	6213.231903	0.711425624		
	BFNTT4	19977.4719	12295.31894	1.624803065		
	BFNTT8	25771.23085	12699.44504	2.029319453		
	BFNTT9	22880.01186	26089.7276	0.876973965		
	BFNTT10	21762.32749	11823.28136	1.840633477	1.416631	.5888644

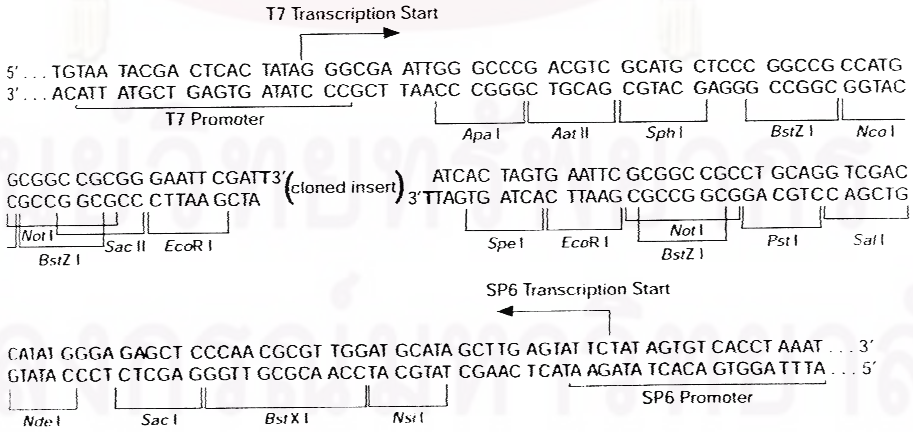
Table. A5 Relative expression level data of *26S proteasome regulatory subunit S3* in testes of male broodstock *P. monodon* using real-time PCR

Sample Group		concentration		Ratio of gene/*EF-1α*	Average	STD
		26S proteasome regulatory subunit S3	*EF-1α*			
1	jntt2	61.64877711	6765.314765	0.009112477		
	jntt3	241.0240673	1338.575648	0.180060102		
	jntt7	383.8083642	3071.112285	0.124973732	.10472	.087256
2	BU10MTT2	21261.08885	66419.71637	0.320102072		
	BU10MTT12	5430.574208	45364.3321	0.119710221		
	BU10MTT13	6985.251515	26947.87685	0.259213427		
	BU10MTT20	2489.74777	23679.63339	0.105143003	.20104	.105468
3	BU14MTT17	19187.62989	31196.7237	0.615052724		
	BU14MTT22	70531.70023	53056.88495	1.329359994		
	BU14MTT37	90449.0423	81193.44963	1.113994327	1.01947	.366415
4	BU18MTT5	356.3999201	6005.59525	0.059344645		
	BU18MTT13	972.8234428	4272.621432	0.227687722		
	BU18MTT14	249.4190915	2680.336283	0.093055149		
	BU18MTT20	318.9981623	11665.81132	0.027344704		
	BU18MTT24	1246.770733	27597.25655	0.045177343	.09052	.080375
5	BFNTT1	304.8659765	6494.519511	0.046942037		
	BFNTT4	10245.18157	13594.25969	0.753640272		
	BFNTT8	13928.10534	14306.69564	0.973537544		
	BFNTT9	7234.364999	13050.72718	0.554326583		
	BFNTT10	32089.1418	27789.65349	1.154715435	.69663	.427819

APPENDIX B

Restriction mapping of pGEM® T-easy Vector





BIOGRAPHY

Miss Sasithon Petkon was born on July 5, 1982 in Khonkaen. She graduated with the degree of Bachelor of Science (Biotechnology) from the Department of Science, Ramkhamhaeng University in 2004. She has enrolled a Master degree program at the Program in Biotechnology, Chulalongkorn University since 2007.

Publications related with this thesis

1. **Petkhon, S.,** Leelatanawit, R., Klinbunga, S. and Menasveta, P. (2009). Cloning and Expression Analysis of Genes in Testes of the Giant Tiger Shrimp *Penaeus monodon*. Proceeding in the 21th Annual Meeting and International conference of the Thai Society for Biotechnology, 24–25 september 2009, Queen Sirikit National Convention Center, Thailand (Poster presentation).