THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Veterinary Parasitology)

DEGREE

Veterinary Parasitology	Parasitology
FIELD	**DEPARTMENT**

TITLE: Proteomic Analysis of Midgut Proteins Associated with Immunity of Thai Cattle Ticks, *Rhipicephalus* (*Boophilus*) *microplus*

NAME: Mr. Sinsamut Saengow

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Associate Professor Sathaporn Jittapalapong, Ph.D.)

COMMITTEE MEMBER

(Assistant Professor Sirichai Wongnakpech, Ph.D.)

COMMITTEE MEMBER

(Mr. Sittiruk Roytrakul, Ph.D.)

COMMITTEE MEMBER

(Associate Professor Win Chaeychomsri, Ph.D.)

DEPARTMENT HEAD

(Associate Professor Sathaporn Jittapalapong, Ph.D,)

APPROVED BY THE GRADUATE SCHOOL ON

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

PROTEOMIC ANALYSIS OF MIDGUT PROTEINS ASSOCIATED WITH IMMUNITY OF THAI CATTLE TICKS *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS*

SINSAMUT SAENGOW

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Veterinary Parasitology)
Graduate School, Kasetsart University
2010

Sinsamut Saengow 2010:Proteomic Analysis of Midgut Proteins Associated with Immunity of Thai Cattle Ticks, *Rhipicephalus* (*Boophilus*) *microplus.* Master of Science (Veterinary Parasitology), Major Field: Veterinary Parasitology, Department of Parasitology. Thesis Advisor: Associate Professor Sathaporn Jittapalapong, Ph.D. 235 pages.

The cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, is an ectoparasite and vector of important blood parasites in cattle such as *Babesia spp* and *Anaplasma spp*. The economic losses of tick infestations in livestock development were effected by the reduction of cattle body weight, and production, as well as chemical, labour, equipment and production losses associated with acaricidial control. The alternative tick control was vaccination against ticks by using candidate proteins such as Bm86 and Bm95 expressed in tick's midgut. The objective of this study was to demonstrate the proteomic profile of tick's gut proteins associated with host's immunity of Thai cattle ticks (*R. microplus*). The result of sodium dodecyl sulfate - polyacrylamide gel electrophoresis and Western blot analysis demonstrated the related protein bands. Nine tick's gut proteins were found by Coomassie staining correlated with immunoblotting. Six and five bands were also found in Bm95 and Bm86, respectively. The result of Liqued Chromatography – Mass Spectrometry analysis showed the most frequently found proteins in each band which was identified as reactive immunogen proteins. These proteins were represented by the highest 50 protein types which anlysed from Mascot MS/MS software. The proteomic analysis of tick gut's proteins revealed the perstective proteins which were indentical to Bm95 and Bm86 such as angiotensin – converting enzyme, SCO – spondin, and serpin. Additionally, these proteins were the member of proteolytic enzyme for example cathepsin L – like cysteine proteinase, OTU – like cysteine protease and amiopeptidase N protein. These proteins might be associated to host – parasite interaction and expected to be the candidate vaccine in the future.

____________________ ____________________ ___ / ___ / ___

Student's signature Thesis Advisor's signature

ACKNOWLEDGMENTS

For the accomplishment of my thesis investigation, I am thoroughly sincerely to my committee members.

To Assoc. Prof. Dr. Sathaporn Jittapalapong, my thesis advisor, for his important advocacy, valuable guidance, and generosity to me for completely performance of my thesis.

To Assist. Prof. Dr. Sirichai Wongnakpech, for his motivation advice , encouragement and valuable recommendation for thoroughly my thesis data with benefit introduction to accomplishment my research.

To Assoc. Prof. Dr. Win Chaeychomsri for his benevolence commendation, inspiration counsel, and considerable information to make my thesis successfully.

To Dr. Sittiruk Roytrakul, for being my ultimate mentor, encouraging and his personal guidance have provided a useful basis for the complete my thesis, charitable and facilities throughout my graduate.

This study was made plausible and conveniently by the most bountiful facilities provide at the Section of Proteomic Laboratory, Department of Parasitology, Faculty of Veterinary, Kasetsart University. The staff of this institution gave me the most sympathy assist in everything. I do thank the laboratory colleagues whom I received their continuous helps and kind suggestions.

This study was supported by a funding from the Graduate School, Kasetsart University.

Finally, I sincerely grateful to my parent who always give me the chance to do everything, cheerfulness, wish well, valuable recommendation and spiritually support.

Sinsamut Saengow
March, 2010

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

Appendix Table

LIST OF TABLES (Continued)

Appendix Table **Page**

LIST OF TABLES (Continued)

Appendix Table **Page**

LIST OF TABLES (Continued)

Appendix Table **Page**

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

Appendix Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES(Continued)

LIST OF ABBREVIATIONS

°C = Degree(s) Celsius

µg = Microgram(s)

µl = Microliter(s)

µm = Micrometer(s)

bp = Base pair(s)

DNA = Deoxyribonucleic acid

dNTP = Deoxynucleotide triphosphate

DW = Distilled water

EDTA = Ethylenediamine tetraacetic acid

ELISA = Enzyme linked-immunosorbent assay

et al = *et alli*

g = Gram(s)

kDa = Kilodalton(s)

kg(s) = Kilogram(s)

L (l)= = Liter(s)

M = Molar

mAmp = Milliampere(s)

mg = Milligram(s)

min = Minute(s)

ml = Milliliter(s)

mm = Millimeter(s)

mM = Millimolar(s)

LIST OF ABBREVIATIONS (Continued)

mRNA = Messenger ribonucleic acid

MW = Molecular weight

ng = Nanogram(s)

nm = Nanometer(s)

OD = Optical density(-ies)

PCR = Polymerase chain reaction

pH = Negative logarithm of hydrogen ion activity

RNA = Ribonucleic acid

RNase = Ribonuclease

rpm = Round(s) per minute

TBE = Tris – boric acid - EDTA

U/µl = Unit(s) per microliter

U/g = Unit(s) per gram

UDW = Ultrapure distilled water

V = Volts

v/v = Volume by volume

w/v = Weight by volume

w/w = Weight by weight

PROTEOMIC ANALYSIS OF MIDGUT PROTEINS ASSOCIATED WITH IMMUNITY OF THAI CATTLE TICKS *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS*

INTRODUCTION

Ticks are obligate blood – feeding ectoparasites that infest numerous species of mammals and are one of the most important vectors in the transmission of human and livestock diseases (Mulenga *et al*., 2000; Jaworski, 2003). The cattle tick, *Rhipicephalus (Boophilus) microplus*, the most prevalent ectoparasitic arthropod of livestock in Central and South America, Australia, Asia, and Africa (Andreotti *et al*., 2000) is a vector of protozoan tick borne diseases such as *Babesia spp*., *Anaplasma spp.* and *Theileria spp*.

Despite the enormous importance of cattle ticks in disease transmission, there are the economic loss by the tick effect on body weight of cattle. A female cattle tick is capable of feeding as much as 1.5 ml of blood. It has been estimated that cattle in tropical areas of Australia may become infested with 1000 tick larvae per day, resulting in greatly reduced productivity (Rand *et al*., 1989). Conventional control are acaricide applications. However, the use of acaricide has several drawbacks including expense, environmental contamination and residues in livestock products. The costs of chemical, labour, equipment and production losses associated with treatment, the cost of maintaining tick boundaries by the state, and possibility of restrictions on the access to market due to the residues have to be considered (Jonsson, 1997). The ability to manipulate organisms on the molecular level and the advent of modern immunological procedures have provided potentially new alternative for tick control. Control of tick by vaccination would have the benefits of being cost – effective, avoid environmental contamination with insecticide and prevent drug – resistant ticks that result from repeated acaricide application. Development of vaccines against ticks would also permit for inclusion of multiple antigens that could target a broad range of tick species and may also prevent transmission of pathogen (de la Fuente and Kocan, 2003)

Many mammals were infested by ticks acquire immunity to subseqeuent infestations, a phenomenon known as tick immunity. Trager (1939) was the first to demonstrate the acquire of resistance to ixodid ticks in quinea pigs and suggested the resistance was based on an immunological basis. The isolation and characterization of the protective antigen required different approach. Willadsen *et al*.(1989) used gel filtration to separate and affinity chromatography to isolate a glycoprotein with a molecular mass of 89,000 dalton (Bm86) from *R. microplus* adult extracts. It was shown that cattle immunized by a membrane – bound glycoprotein (Bm86) were highly resistant to tick infestations (Willadsen *et al*., 1989). A vaccine based on Bm86 was a very attractive and alternative to acaricide treatment since this would overcome most of the difficulties associated with the use of chemicals (Rand *et al*., 1989).

An alternative vaccination approach was the use of tick’s gut antigen, as the targets of the immune response. As *R. microplus* is a one – host tick, it must be coped with the pressure of the immune response resulting from the feeding of all stages on a host. For the Bm86 –derived tick vaccine, antibodies bind to antigenic epitopes on the midgut cells of the feeding tick causing damage and leakage of blood into the body cavity, killing the tick or reducing fecundity (Rand *et al*., 1989). However, previous investigators have concluded that the reduced efficacy was due to Bm86 heterogeneity among *R. microplus* strains (Garcia – Garcia *et al*., 1999). An ideal ectoparasite vaccine would be incorporated the ‘concealed antigen’ approach while inducing an anamnestic response following natural infestation, thus eliminating the need for repeat vaccination (Trimnell *et al*., 2002).

Midgut has been used as an immunogen (Jackson and Opdebeeck., 1990; Opdebeeck *et al*., 1988 b.). Studies on the midgut of ticks are important since it is the site for the development of egg transformation and is the major physical barrier between ticks and host defense mechanism (Agyei and Runham, 1995). For the success in the development of commercial Bm86 vaccine against the cattle tick, the understanding of midgut protein associated with immunity is necessary. In improvement of advance vaccine against cattle tick, various conceal gut antigen form *R. microplus* have been identified viz, Bm86, Bm91, Bm95, BmPRM, etc., which have been used alone or in combination (de la Fuente *et al*., 2006b.; Nuttall *et al*., 2006). Consequently by Bm95 presented the good promise and has shown to induce protection event against Bm86 – resistant *R. microplus* strain (de la Fuente *et al*., 2000).

Thus, the discovery of tick protective antigen has been the limiting step in the development of tick vaccine. Although several tick proteins have been proposed as putative protect antigens, few have been tested as recombinant protein in experimental vaccination (de la Fuente and Kocan, 2003; Willadsen, 2004). Among them, the *R. microplus* gut proteins, such as Bm86 and Bm95 gut proteins, were not much information in function but they have affected tick infestations (de la Fuente *et al*., 2006a.). Therefore, this study is demonstrated the midgut proteins from Thai cattle tick, *R. microplus*, that can induce the host immune response against cattle ticks.

OBJECTIVES

To demonstrate proteomic profiles of *R. microplus*’s midgut proteins associated with host immunity.

LITERATURE REVIEW

Taxonomy and Morphology

The cattle tick, *Rhipicephalus (Boophilus) microplus,* is an ectoparasite in Family Ixodidae, Genus *Boophilus*, and belong to Phylum Arthoropoda. *R. microplus* is classified as a one – host tick which passes through three life cycle process in one host (Sutherst *et al*., 1978). However, *Boophilus* has recently become a subgenus of the genus *Rhipicephalus*. *Boophilus* ticks have a hexagonal basis capitulum. The spiracular plate is rounded or oval and the palps are very short, compressed and ridged dorsally and laterally. Males have adenal shields and accessory shields. The anal groove is absent or indistinct in females, and faint in males. There are no festoons or ornamentation (Institute for International Cooperation in Animal Biologics, Iowa State University, 2007).

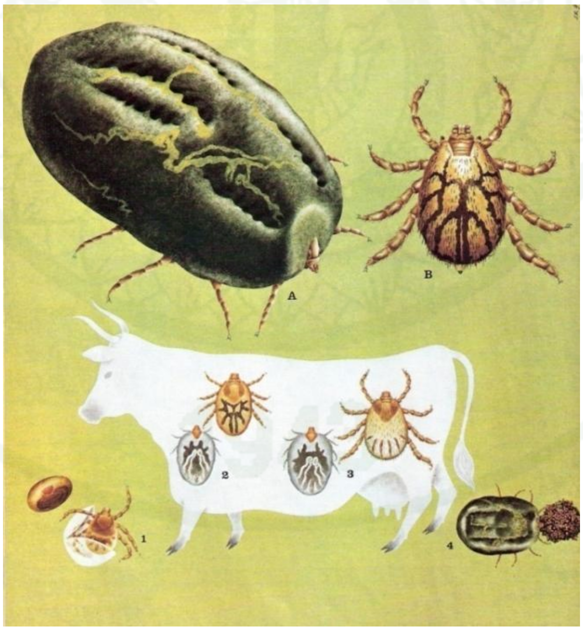


Figure 1 The life cycle of the one – host tick, *Boophilus* spp

Source: Cooper (1977).

Life Cycle

R. microplus is a one-host tick; all stages are spent on one animal. The eggs hatch in the environment and the larvae crawl up grass or other plants to find a host. They may also be blown by the wind. In the summer, *R. microplus* can survive for as long as 3 to 4 months without feeding. In cooler temperatures, they may live without food for up to six months. Ticks that do not find a host eventually die of starvation (Institute for International Cooperation in Animal Biologics, Iowa State University, 2007).

Newly attached seed ticks (larvae) are usually found on the softer skin inside the thigh, flanks, and forelegs. They may also be seen on the abdomen and brisket. After feeding, the larvae molt twice to become nymphs and then adults. Each developmental stage (larva, nymph and adult) feeds only once, but the feeding takes places over several days. Adult male ticks become sexually mature after feeding, and mate with feeding females. An adult female tick that has fed and mated detaches from the host and deposits a single batch of many eggs in the environment. Typically, these eggs are placed in crevices or debris, or under stones. The female tick dies after ovipositing. *Boophilus* has a life cycle that can be completed in 3 to 4 weeks and this characteristic can result in a heavy tick burden on animals.

After hatching, *R. microplus* larvae find their vertebrate hosts. During the following 3 weeks, the larva feeds on small amounts of blood and after engorgement, they molt to nymph that do the same process to become adults. The adult female ingests blood equivalent to approximately 100 times its own body mass. Being a single host, the engorged female drops from the bovine and dies approximately 1 month later (Lara *et al.*, 2003) after lying 3,000 – 4,000 eggs.

Veterinary Important

I. Tick – borne diseases

The cattle tick, *R. microplus* is one of the most important arthropod pests in livestock in Central and South America, Australia, Asia, and Africa (Andreotti *et al*., 2000). This tick is a natural vector of many parasitic protozoa and ricketsia such as *Babesia* spp. caused of babesiosis and *Anaplasma marginale* caused of anaplasmosis in bovine (Untalan *et al*., 2005). In Cuba alone, babesiosis and anaplasmosis have caused more than 100,000 death of cattle over the last decade (Serrano *et al*., 1995).

II. Tick infestation

With other hematophagous arthropod vectors of disease, ticks also cause of economic loss to livestock by their effects on bodyweight of cattle, and transmission of tick fever, produce weakness, reduce milk and meat production (Boué *et al*., 1999). Each engorged female tick has been shown to reduce host weight gain by 0.6 g in beef cattle, of which 65% has been attributed to inappetence induce by infestation; so it is likely that infestation has substantial effect on milk production (Sutherst *et al*., 1983; Seedbeck *et al*., 1971).

III. Economic losses

Ticks are normally controlled by regular acaricides that have their costs including chemical substances, labors and equipment. Moreover, production losses associated with treatment, the cost to the state of maintaining tick boundaries, and the possibility of restriction on the access to markets due to residues have be considered (Jonsson, 1997). The costs associated with production losses and control of the parasite in Australia have been estimated to exceed $ 100 million per annum (Cobon and Willadsen., 1990) and $ 1 billion in South Africa (Horn 1987). Annually, ticks caused serious economic losses to animal production, closed to a billion of dollar (Jonsson, 1997).

Tick Control

Conventional Control

Traditionally, tick control has been done by the use of chemical, beginning with arsenic in the 19th century and proceeding through Dichloro Diphenyl Trichloroethane (DDT) and other organophosphate derivatives, organocarbamates, amidines, and synthetic pyrethroides. Resistance by ticks had been developed (Nolan *et al*., 1986, 1989; Schinitzerling *et al*., 1989) and increasing in tick populations. Moreover the presence of chemical residues in milk and meat are concerned that emphasize the need for novel control method, such as vaccination and biological control (Willadsen *et al*., 1996; Gindin *et al*., 2002).

Market forces are creating two conflicting demands, first, for the increased use of breeds of cattle which are tick – resistance to reduce the use of chemical agents. Due to the increasing trend of organic food, the usefulness of many chemical agents is also declining and the desirability of alternative, environmentally friendly technology, such as immunological control, is well demanded (Willadsen *et al*., 1995). Additionally, in the context of animal production, vaccine is an attractive strategy because they are safe for the environment, for the host, and for the consumers (Kashino *et al*., 2005).

Anti - Tick Vaccination**:** *Rhipicephalus* (*Boophilus*) *microplus*

The hypothesis to make the vaccination against – tick was initiated in 1939 (Trager, 1939). Vaccination with concealed antigen was proposed by Galun (1978) who demonstrated study indicated that ingested components of the host immune system in the tick gut might damage parasite organ not normally exposed to the host.

Johnson *et al* (1986) have been reported that vaccination using gut antigen of cattle against cattle tick, *R. microplus* led to a 70 % reduction in tick fecundity . Furthermore, subsequently observation on vaccinated cattle demonstrated that host –

immune responses had damaged the tick's gut up to 60% of the female tick (Agbede and Kemp, 1986). Additionally, cattle vaccination with different organ extraction from whole *R. miroplus*, semi – engorged female ticks, midgut extract, or larval extract, shown the significant decreases in reproductive parameters and tick number (Opdebeeck *et al*., 1988(a) – 1989; Willadsen *et al*., 1988; Wong and Opdebeeck, 1989). After the discovery of midgut protein, Bm86, the commercial vaccine against *R. microplus* was developed, distributed worldwide, and initiated the study of other candidate immunogens of *R. microplus* vaccine in cattle.

Tick Vaccination

Commercial Vaccine

A protective antigen, Bm86 was subsequently identified, purified from tick extracts (Willadsen *et al*., 1989), and expressed using recombinant DNA system (Willadsen, 1990; Rodríquez *et al*., 1994). The protective mechanism of these vaccines is the production of antibodies against protein localized in the midgut and acts directly on the tick gut reducing the number of engorging ticks, the weight of engorged females, and their fecundity (Willadsen *et al*., 1989; de la Fuente *et al*., 1998).

In 1993 and 1994, vaccine against the cattle tick *R. microplus* containing the recombinant Bm86 antigen were registered in Cuba, (Gavac™, Herber Biotec S.A., Havana, Cuba) and Australia (TickGARD, Hoechst Animal Health, Australia), respectively. The effect of these vaccines is not directly killing ticks, but it has an effect on a successive reduction in tick numbers (Rodríquez *et al*., 1995(b)).

Table 1 Anti-Bm86 antibody titer parameters for different cattle breeds vaccinated with GavacTM

Cattle breed	Location	*Ti*	τ (Week)	*Tb*	Reference
Holstein cross – bred herd 1	Limonar, Matanzas, Cuba	48,000	7.5	48,000	Rodriguez *et al*., 1995a Labarta *et al*., 1996
Holstein cross – bred herd 2	Los Narajos, Havana, Cuba	8,194	27	8,194	Rodriguez *et al*., 1995b
Guzera x Jersey and Gir x Holstein	Bnanal, Sao Paulo, Brazil	16,000	17	16,000	Rodriguez *et al*., 1995c
B. indicus x Simmental; *B. indicus* x Beefmaster; and *B. indicus* x Charolaise	Tamaulipas, Mexico	3,555	26	8,140	Redondo *et al*., 1999

Ti, initial maximum titer; τ, titer half – life, *Tb*, booster maximum titer.

Source: Lodos *et al*. (2000)

Table 2 Maximum thresholds for accumulated damage coefficients of engorged females (*S*f) and the number of eggs laid per female (*S*e) for different tick strains

Tick Strain	Location	Sf	St	Reference
Camcord	Limonar, Matanzas, Cuba	35,000	0	Rodriguez *et al*., 1995a; Labarta *et al*., 1996
Camcord	Los Naranjos, Havana, Cuba	10,561	0	Rodriguez *et al*., 1995b
Field strain	Bananal, Sao Paula, Brazil	25,389	0	Rodriguez *et al*., 1995c
La Mora	Tanaulipas, Mexico	7,537	7,537	Redondo *et al*., 1999

Source: Lodos *et al*. (2000)

Recently, there is a new technique using immunological methods for the identification, isolation, and synthesis of protein that attack tick tissues and organs;

mainly those of the reproductive system (Tellam *et al*., 1992; Willadsen, 1997). Additionally, the present about the tick ovary was aimed to understand the morphological, histological, and ultrastructural features of the female reproductive system of *R. microplus* as well as to attempt to establish the dynamics of the vitellogenensis process to provide information that would contribute to the future control of this parasite (Saito *et al*., 2005).

Salivary Protein for Candidate Vaccine

Salivary gland and saliva – secretion performed a important characterization during host – tick interaction since this organ is responsible for tick fluid hemostasis and the secretion of molecule that evading host immune mechanism (Ribeiro, 1995). And also, they are particularly the main transmission route for pathogenic organism (Sauer *et al*., 1995; Nunes *et al*., 2005).

Salivary gland has shown the morphological and functional change during the development of physiological stage of tick. The structure of this organ during the proliferative phase was massive acini, secretory cell with granules of cytoplasmic secretion and large nuclei (Schumaker and Serra – Freire, 1991) followed by a gradual regression with morphological and structural changes including autolysis (Fawcell *et al*., 1986). According to the complete engorgement phase, the tick's salivary gland become a decreasing of size (in argasid) or degeneration (Till, 1961).

During a blood meal, ticks ingest large amounts of host serum containing immunoglobulins, which makes antibodies logical candidates for mediating the expression of the distinct phenotypes of tick infestations. Antibodies may confer resistance by neutralizing the salivary function. Once host blood is ingested, damaging the parasite's tissues will be happened (Kashino *et al*., 2005). Furthermore, depending on their characteristics, antibodies can mediate the inflammatory reactions of the host (Casadevall and Pirofski., 2003) that are detrimental to ticks (Ribeiro, 1995; Wikel, 1996).

Midgut Protein for Candidate Vaccine

The digestion of blood meal plays an essential role in the biology of the tick, such as molting or vitellogenesis follow blood meals provide energy and mass change from the nutrient (Grandjean, 1984). Studies on the midgut of ticks are important for many reasons since it is the site for the development of blood – borne parasites transmitted by the tick and secondly the midgut and its epithelial lining is a major physical barrier between ticks and host defense mechanism. The location of the site of development of these blood – borne parasite is the fundamental importance since its helps in the understanding of the relationship between the host and its parasite (Agyei and Runham, 1995).

Midgut has been used as an immunogen (Opdebeeck *et al*., 1988 a&b; Jackson and Opdebeeck., 1990) but its feasibility as a vaccine required the production of the antigen material in large quantity (Akhtar *et al*., 1992).

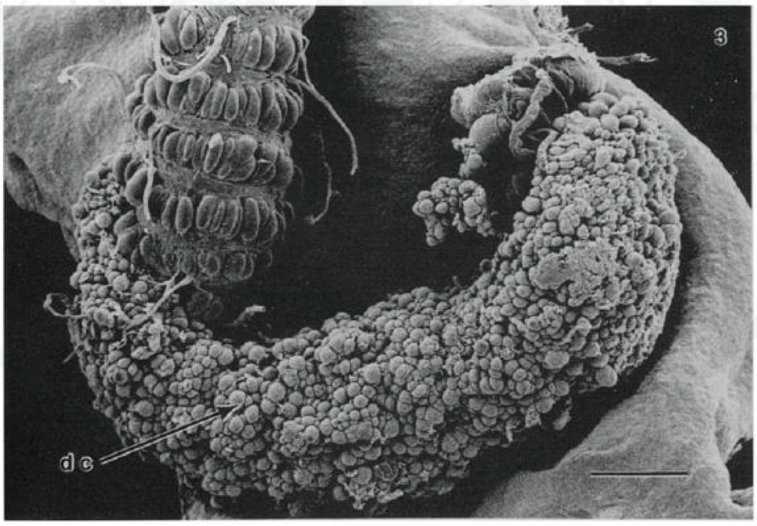


Figure 2 Scanning electron micrograph of dissected tick gut with one end everted to expose digest cells (dc). Scale bar = 400 µm.

Source: Kemp *et al*. (1989)

For mid gut immunogen, it was shown that cattle immunized against a membrane – bound glycoprotein (Bm86) purified from cattle tick are highly resistance to parasite challenge. The effects are very striking as many as 90% of ticks failed to survive (Willadsen *et al.,* 1989). However it was necessary to begin with 40,000 – 60,000 ticks in order to get 20 – 100 µg of Bm86. To produce the larger quantities of Bm86 there are needed to clone and express the Bm86 gene. Recombinant Bm86 protein is capable of inducing a substantial degree of protection against tick infestation (Rand *et al.*, 1989).

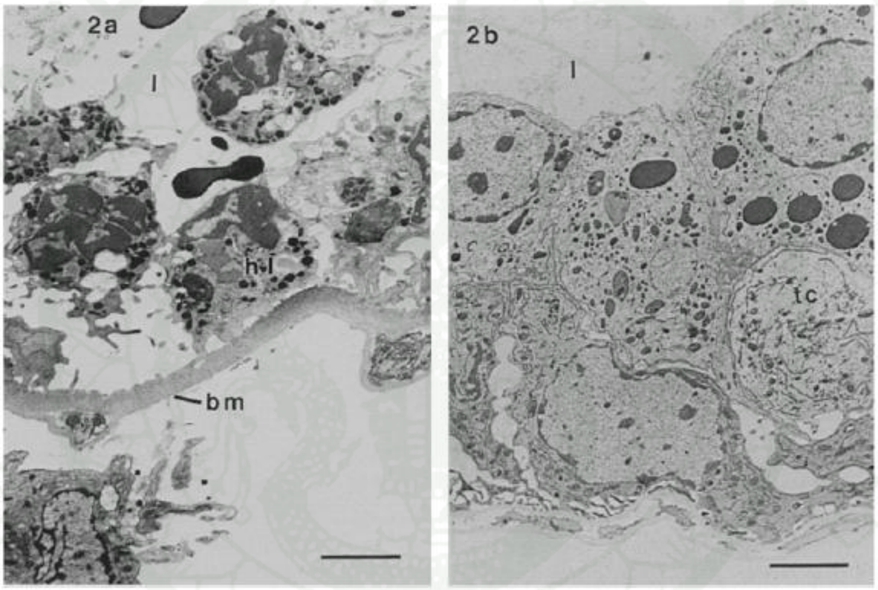


Figure 3 Comparison of female tick gut in taken from vaccinated (2a) and control (2b) cattle. On vaccinated cattle, the tick cells (tc) have been lysed, and host leucocyte~ (hl) appear to be attached to the gut basement membrane (bin). Gut lumen (1). (2a) Scale bar = 4 µm. (2b) Scale bar = 10 µm.

Source: Kemp *et al*. (1989)

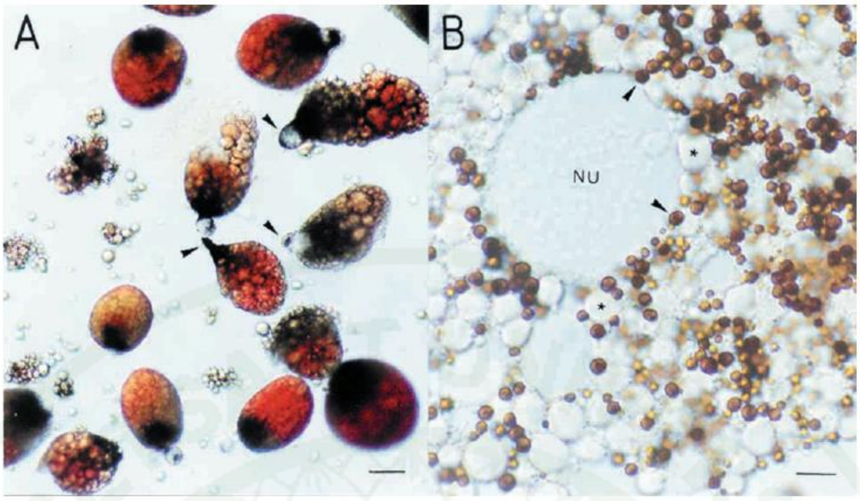


Figure 4 Digest cells on the third day after blood meal, isolated and observed by differential interference contrast microscopy. (A) Digest cells separated from the midgut were observed without a cover slip. Arrowheads show the site of attachment to the gut epithelium (scale bar, 55·mm). (B) Digest cells observed with a coverslip, at higher magnification. Hemosomes are concentrated in the perinuclear area (arrowheads). Asterisks indicate digestive vesicles (scale bar, 5·mm. NU, nucleus).

Source: Lara *et al*. (2003)

Midgut antigens are the promising target of the advancement of the vaccine strategies because antigens on the luminal surface of the midgut are directly exposed to the blood meal and host immune effectors. Since blood meal digestion in ticks is intracellular (Sonenshine, 1991), host immunoglobulins may also contact intracellular midgut antigen. Midgut antigens of *R. microplus* have previously been exploited as targets for vaccine development. For example, the midgut protein Bm86, which has been shown to reduce survival and reproduction rates of ticks, has been the basis for a commercial vaccine (Willadsen, 2004). Glycosylation of tick proteins appears to be important for increasing the protective capacity of tick vaccine antigen preparation, as shown in the investigation with yeast – derived recombinant Bm86 and Bm95 proteins (de la Fuente *et al*., 2006(a)). Insignificant differences in the nucleotide sequence of homologous Bm86 genes isolated from different tick strains can have an effect on how efficiently an antigen protects cattle. It was presented that a Bm95 homologue of Bm86 could yield a more universal vaccine, which might be able to protect cattle against *R. microplus* strains form different geographical regions (Garcia – Garcia *et al*., 2000)

Proteomic Analysis

Proteomic technology in general deals with the large – scale determination of gene and cellular function directly at the protein level (Aebersold and Mann., 2003) and consisted of genomic sequencing, microarray analysis and metabolite profiling (Patterson and Aebersold., 2003). The essence of this emerging system biology approach is shown in table 3. Proteomic is a particularly rich source of biological information because proteins are involved in almost all biological activities and they also have diverse properties, which collectively contribute greatly to our understanding of biological system (Patterson and Aebersold., 2003).

Current goals of proteomic research are more varied and directed toward the systematic determination of diverse properties of proteins. These include sequence, quantify, state of modification, interaction with other protein, activity, subcellular distribution and structure (Patterson and Aebersold., 2003). The current interest in proteomic is due in part to the prospects that a proteomic approach to disease investigations will overcome some of the limitations of other approaches (Petricoin *et al*., 2002).

Polyarylamide Gel Electrophoresis and Western blot technique

Polyacrylamide gel electrophoresis (PAGE) is a premier tool for the analysis of protein molecular weight. Protein (like nucleic acids) possess a charge and thus migrate when introduced into an electric field. Protein are denatured and electrophoresed through a matrix of acrylamide that is inert (so that protein can move through it). The velocity of a protein as it migrates through an acrylamide gel is inversely proportional to its size and thus a complex mixture of protein can be separated in a single experiment. Proteins are almost always electrophoresed through arylamide under denaturing conditions in the presence of the detergent sodium dodecyl sulfate (SDS), so this technique is commonly abbreviated SDS – PAGE (Pevsner, 2003).

O’ Farell (1975) greatly extended the capabilities of this technology by combining it with an initial separation of protein based on their charge. In the first step, proteins are separated by isoelectric focusing. A gel matrix (or strip) is produced that contains ampholytes spanning a continuous range of pH values, usually between pH3 and 11. Each protein is zwitterionic and when electrophoresed, it migrates to the position at which its total net charge is zero. This is the isoelectric point (abbreviated pI) at which the protein stops migrating. A complex mixture of protein may thus be separated based upon charge, and this corresponds to the first dimension of two – dimensional gel electrophoresis. In the second dimension, protein are separated by SDS – PAGE. The technique of two – dimensional gel electrophoreis has matured into an important technology used to analyze proteomes (Dunn, 2000).

Western Blotting

Western blotting (Towbin *et al*., 1979; Towbin and Gordon., 1984) is to proteins what Southern blotting is to DNA. In both techniques, electrophoretically separated components are transferred from a gel to a solid support and probed with reagents that are specific for particular sequences of amino acids (western blotting) or nucleotides (Southern hybridization). In the displayed by the target protein attached to the solid support. Western blotting is therefore extremely useful for the identification and quantification of specific protein in complex mixtures of proteins that are not radiolabeled. The technique is almost as sensitive as standard solid – phase radioimmunoassay and unlike immnunoprecipitation, does not require that the target protein be radiolabeled. Furthermore, because electrophoretic separations of proteins is almost always carried out under denaturing conditions, any problem of solubilization, aggregation, and coptecipitation of the target protein with adventitious proteins are eliminated (Sambrook and Russell, 2001).

In western blotting, the sample to be assayed are solubilized with detergents and reducing agents, separated by SDS – polyacrilamide gel electrophoresis, and transferred to a solid support (usually a nitrocellulose or PVDF filter), which may then be stained (e.g. with Ponceau S). The filter is subsequently exposed to unlabeled antibodies specific for the target protein. Finally, the bound antibody is detected by one of several secondary immunological reagent, followed by autoradiography, enhanced chemiluminescence, or enzymatic production of a colored precipitate. As little as 1 – 5 ng of an average – sized protein can be detected by western blotting (Sambrook and Russell, 2001).

Table 3 Glossary of experimental terms used in proteomics.

	Glossary of Experimental terms used in proteomic
2DE	Two – dimension gel electrophoresis is the separation of protein using two orthogonal parameters, isoelectric point (charge) and relative molecular mass, which are both usually determined on the basis of protein mobility in a polyacrylamide gel matrix.
Discovery science	Investigation of a biological system or process by enumerating the elements of a system irrespective of any hypotheses on how the system might function.
DNA microarray	A high – throughput differential screen a mRNA expression using complementary cDNA or oligonucleotide libraries that are printed in extremely high density on microchip; the microchips are probed with a mixture of fluorescently tagged cDNAs that are products from two different cell population and analyzed with a laser confocal scanner.

Table 3 (Continued)

Glossary of Experimental terms used in proteomic	
ESI	The electrospray ionization process is achived by spraying a solution (such as the effluent of a HPLC column) through a charged needle at atmospheric pressure toward the inlet of the mass spectrometry; the voltage applied to the needle tip and a pressure differential result in the formation of ions for mass analysis and their transfer into the mass spectrometer.
ICAT	Isotope – coded affinity tag reagent comprising a chemical modifying group linked to an affinity group through a mass – encoded linker.
Ion source	Mass spectrometer component designed to use the principle of an ionization method for generating ions (charged analytes) for mass analysis.
Ionization	Process of adding charge onto uncharged (neutral) analyte, in other words, the formation of an ion; either ionization is conducted in a vacuum or ions formed at atmospheric pressure are transferred into the vacuum system of the mass spectrometer
MALDI	Matrix – associated laser desorption ionization is a process by which ion formation is promoted by short laser pulses; the sample is deposited on a sample plate into the source (which is held under vacuum) and then embedded in a matrix that promotes ionization; a laser fired at the sample that is co – crystallized with the matrix results in the desorption of the analyte from the sample plate and its ionization.
Mass analyzer	Mass spectrometer component that can measure the mass – to – charge ratio of charged molecules (ions); ion – trap, quadrupole and time of flight (TOF) analyzers are used most often.
Mass spectrometry	Accurate mass measurement of charged analytes (ions); in the context of proteomic, analytes are usually peptides or less frequently protein ions; a mass spectrometer measures the mass – to – charge ratio of charged species under vacuum and comprises, broadly, an ionization source and a mass analyzer.

Table 3 (Continued)

Glossary of Experimental terms used in proteomic	
MS spectra	Single – stage mass spectrometry spectra provide mass information on all ionizable component in a samples; these data are used, for example, for peptide mass fingerprinting.
MS/MS spectra	MS/MS spectra are generated in instruments equipped with a mass filter that can select a peptide ion from a mixture of peptide ions, a collision cell in which peptide ions are fragmented into a series of products ions (through collision of the selected precursor ion with a noble gas in a process referred to as CID), and a second mass analyzer that records the fragment ion mass spectrum; the fragment ion spectra are referred to as either MS/MS spectra or CID spectra.
Protein identification	Method to determine the sequence identity of a protein; two common mass spectrometry – based approaches used are peptide mass mapping and uninterpreted MS/MS spectra; in both methods, observed data are matched to theoretically derived peptide and/or fragment ion masses calculated from sequence databases
System biology	Study of biolobical system by the systematic and quantitative analysis of all of the components that constitute the systems.
Yeast two – hybrid	Genetic – based method for identifying protein – protein interaction in vivo; a protein fused to the DNA – binding domain (the ‘bait’) and a (different) protein fused to the activation domain of a transcriptional activator (the ‘pray’) are expressed in yeast cells; if an interaction between the bait and the pray occurs, transcription of a reporter gene is induced and detected typically by a color reaction that indicates transactivation of the reporter gene

Source: Patterson and Aebersold. (2003)

Mass Spectrometry

To measure the mass or more specifically, the mass – to – charge ratio (m/z) of a molecule in a mass spectrometer, the analyte must first be ionized and transferred into the high vacuum system of the instrument. Peptide and protein, like other larger molecules proved difficult to ionize under conditions that did not destroy the molecule. In the late 1980s, two methods were developed that allowed the “ionization” of peptide and proteins at high sensitivity and without excessive fragmentation. These breakthrough were electrospray ionization (‘ESI’) (Fenn *et al*., 1990) and matrix – assisted laser desorption ionization (‘MALDI’) (Karas and Hillenkamp, 1988) which had closely followed the development of laser desorption (Tanaka *et al*., 1987, 1988).

In comparison to the nucleic acid – based counterpart, geonomics, the experimental complexity of proteomics is far greater. The technology is also not as mature and owing to the lack of amplification schemes akin to PCR, only proteins isolated form a nature source can be analyzed. Proteomic analyses are therefore generally limited by substrate. The complexities of the proteome arise because most proteins seem to be processed and modified in complex easy and can be the products of differential splicing; in addition, protein abundance spans a range estimated at five to six orders of magnitude for yeast cell (Gygi *et al*., 1999) and more than ten orders of magnitude for human blood serum – for example, form interleukin – 6 at ~ 2pg/ml (Lai *et al*., 2002)

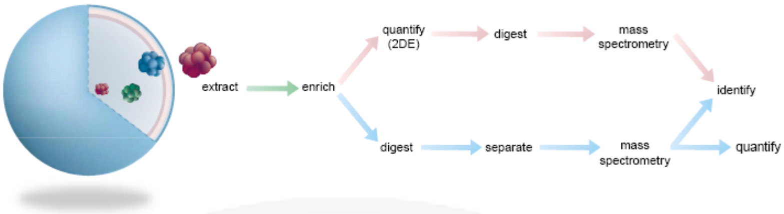


Figure 5 Quantitative identified protein analysis from the cell.

Source: Patterson and Aebersold. (2003).

The increased use of MALDI – MS and ESI –MS/Ms in the identification of 2DE – separated protein showed that the incidence of comigrating proteins even in this, the highest resolving protein method known, was more prevalent than had been originally thought (Gygi *et al*., 2000). Because quantification in 2DE relies on the assumption that one protein is present in each spot, comigration compromises such analyses. It was also observed that with conventional protein staining method only a relatively small subset of a cellular proteome was apparent when unfractionated cell lysates were separated by 2DE (Gygi *et al*., 2000; Corthals *et al*., 2000). Thus, despite the maturity and unmatched performance of 2DE for separating proteins (Gauss *et al*., 1999), and despite the continuing evolution of 2DE separation (Unlu *et al*., 1997;Herbert, 1999; Gorg *et al*., 2000; Rabilloud, 2002) and detection technology (Rabilloud *et al*., 2001), alternative methods for large – scale protein expression analysis began to be investigated more vigorously (Patterson and Aebersold., 2003).

Liquid Chromatography – Mass spectrometry.

Liquid chromatography – mass spectrometry is the analytical chemistry method consolidate of liquid chromatography – mass spectrometry. This combination process can be reconsidered as being one of the most important method of the last decade of twentieth century and was powerful technique used for many application which has very high sensitivity and specific. LC – MS has become the method – of

– choice for analytical support in many stages of drug development within the pharmaceutical industry (Niessen, 1999). Additionally, it performed the considerable character in environmental analysis, as well as in many other field of application.

Liquid chromatography and mass – spectrometry (LC – MS) has been reviewed over the past years, paying attention to instrumental aspects and application (Niessen and Tinke, 1995). Many of the analytes, such as pesticides, drug and toxic substances are suitable for LC – MS analysis, as a consequence of their polarity, thermal lability, or low volatility. Therefore, LC has acquired a role of growing important in food analysis, as is asserted by the wide variety of application reported in recent years. Regarding to LC – MS technique, the advantages achieve by a combination of the separation capabilities of LC and the power MS as an identification and verification method. Using conventional LC detectors, detection may be complicated by false – positive results. In such cases, mass spectrometry has proved to be a valuable technique for the unambiguous identification of contaminants in food (Careri *et al*., 1996)

The application of electrospray ionization (ESI) interfacing has been developed since 1990 (Nissen and Tinke, 1995). The process of electrospray ionization includes both the nebulization of a liquid into a aerosol of highly charged droplets and the ionization of solvated analytic classification after desolvation of the charged droplet. The electrospray process is initiated by applying an electrical potential of several kV to liquid in a narrow – bone capillary or electrospray needle. By increasing the potential difference between the needle and the counter electrode, a series of processes take place. The transition between these processes can be monitored by measuring the current between needle and counter electrode (Nissen and Tinke, 1995).

An important breakthrough in the field of electrospray LC – MS, which gave a vast stimulation to the attraction in the electrospray interfacing development, was the observation in the late 1980s of the multiply charging of peptides and protein by protonation at the amino terminus as well as at the basic amino groups in the molecule, i.e., lysine, arginine, and histidine (Meng et al., 1988; Covey *et al*., 1988). As a result, a statistic charge distribution is observed as an ion enveloped in the region of m/z = 500 – 1500. From the mass spectrum, an accurate calculation (0.01% or better) of the mass for the macromolecule can be accomplished. The application of these protocol resulted in new exiting field of biochemical and biological MS (Fenn *et al*., 1990; Smith *et al*., 1990 – 1991; Carr *et al*., 1991).

Various application of electrospray ionization have been reported. Most of these investigation use electrospray in the characterization of biomolecules, especially protein. The potential of electrospray in such biochemical studies has been reviewed by several authors (Smith *et al*, 1990 – 1991; Carr *et al*., 1991; Mann *et al*., 1990). More recently, a number of LC – MS application with electrospray have been reported (Straub and Vorksner, 1993; Perkin *et al*., 1999). Since with the second generation of electrospray interfaces the allowable flow – rates are more in agreement with flow – rates currently used in LC, a more extended use of electrospray in combination with LC – MS can be predicted for the near feature (Nissen and Tinke, 1995).

In the journal book on LC – MS, Willoughby *et al* (1998) identified the development of LC – MS as well as its acceptance as an analytical technique. The figure 6 demonstrates the model for the distribute of applying LC – MS technique. The significant progress is made in the implementation and application of LC – MS in many different science fields. This phase puts different demands on the development of LC – MS as an analytical technique, as is for instance indicated by the currently growing interest in software development, especially for more efficient data processing after LC – MS analysis.

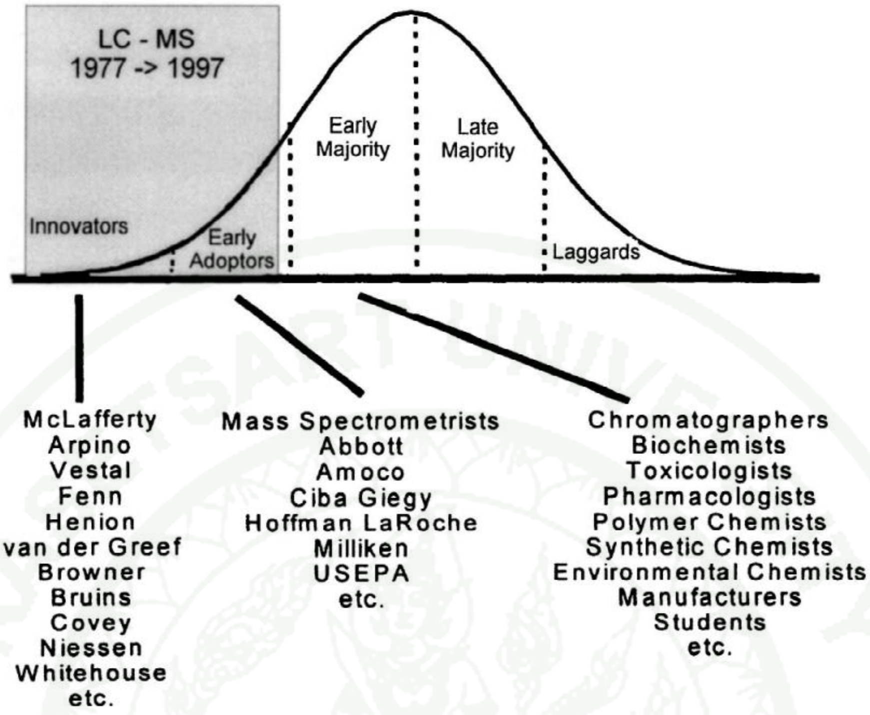


Figure 6 A model for the distribution of people applying LC – MS

Source: Niessen. (1999)

Tick Protein Analysis

For the success in the development of commercial vaccine against the cattle tick, *Rhipicephalus (Boophilus) microplus*, by tick gut glycoprotein, Bm86. The study about the tick organ acts as an immunogen to the host immune system was provided (Willadsen *et al*., 1995). Host immunoglobulin directed against Bm86 during natural tick infestation, and resulting in damage to the tick midgut and inter organs (Rodríquez *et al*., 1994). However, some investigators have concluded that the reduced efficacy is due to Bm86 heterogeneity among *R. microplus* strains, and that efficacy of the Australian Yerrongpilly strain – based vaccine is inversely correlated to variation of a 35 amino acid segment of Bm86 (Garcia – Garcia *et al*., 1999)

Recently, the study about the proteomic of midgut protein presented the identification of 19 proteins that are abundantly expressed during the larval stage of *R. microplus* development. This information was used in the assembly of a two – dimensional (2D) database of expressed larval proteins, which extended the understanding of the repertoire of molecule produced by the tick and aid in the identification of proteins to which the host elicits an immune response at this stage(Untalan *et al*., 2005).

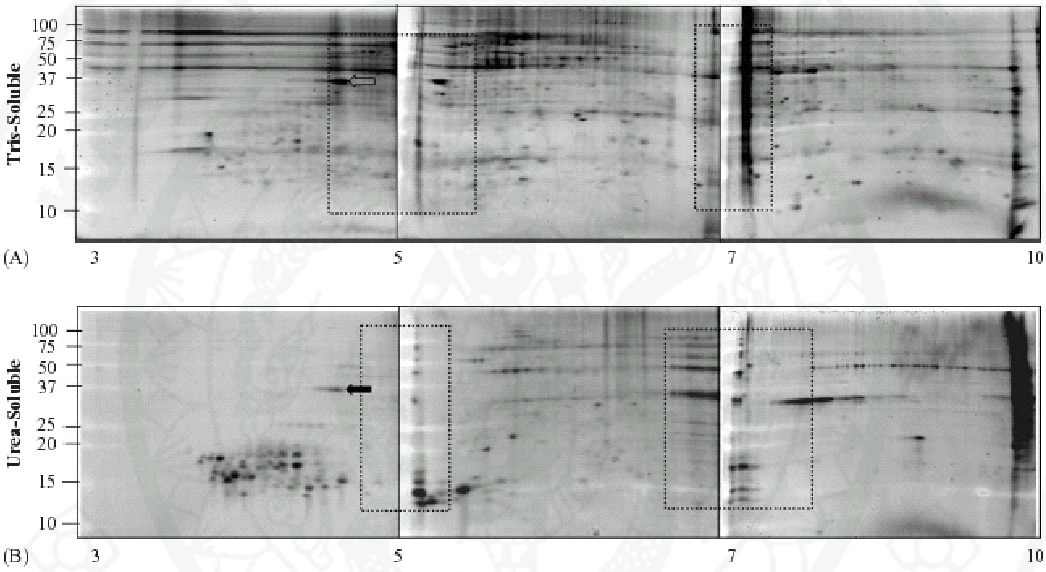


Figure7 Representative 2D gel electropherograms of Tris-soluble (Panel A) and urea-soluble (Panel B) protein fractions isolated from unfed larvae of *R. microplus*. Proteins (50 mg) were separated by IEF (horizontal axis) using 7 cm IPG strips with a pI range of 3–6, 5–8, and 7–10 followed by resolution in the second dimension on 8–16% gradient polyacrylamide gels (vertical axis). Molecular masses in kiloDaltons (kDa) are indicated on the left of each panel. Regions of gel overlap between pI ranges are defined by a thatched box. The white arrow in Panel A highlights the source of Tris-soluble protein carryover into the urea-soluble fraction.

Source: Utalan *et al*. (2005)

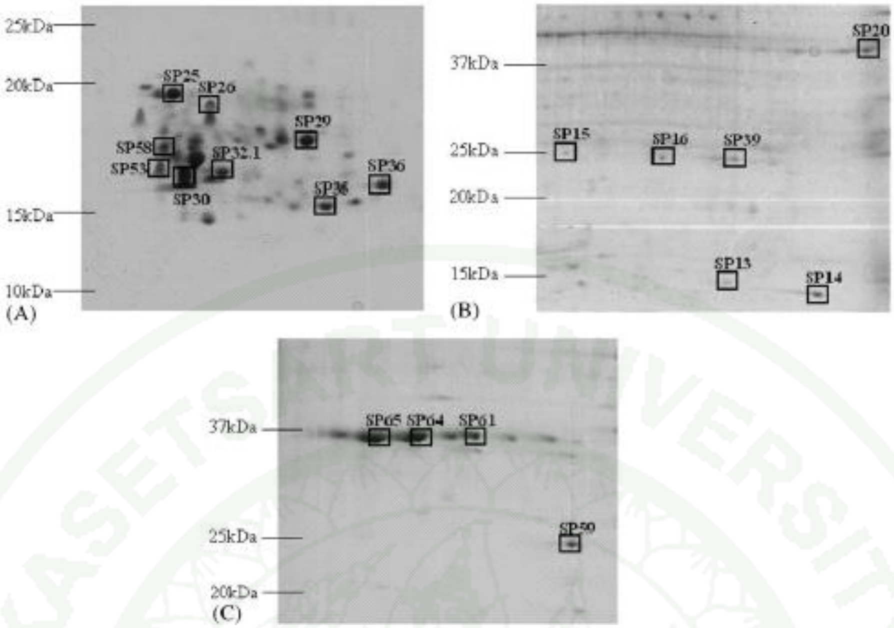


Figure8 High resolution 2D gel separation of urea- (Panel A and C) and Tris- (Panel B) soluble proteins for MS analysis. Proteins isolated from larvae were separated in the first dimension by IEF using 17 cm IPG strips (Panel A: 3–6, Panel B: 5–8, and Panel C: 7–10) and in the second dimension by large format 8–16% gradient gels. Molecular masses in kiloDaltons (kDa) are indicated on the left of each panel. The proteins identified by a square and 2D spot number were selected for subsequent protein identification by MS.

Source: Untalan *et al*. (2005)

Thus, the discovery of tick protective antigen has been the limiting step in the development of tick vaccine. Although several tick proteins have been proposed as putative protect antigen, few have been tested in vaccination experimental as recombinant protein (de la Fuente and Kocan, 2003; Willadsen, 2004). Among them, the *Rhipicephalus (Boophilus) microplus* gut proteins, such as Bm86 and Bm95 gut proteins , was unknown function but they have affected tick infestation (de la Fuente *et al*., 2006(a)). Therefore, this study is attempted to determine the midgut proteins from Thai cattle tick, *R. microplus*, that can induced the host immune response against the tick.

MATERIALS AND METHODS

The experimental method was done as described by the flow chart 1 as demonstrated in figure 9.

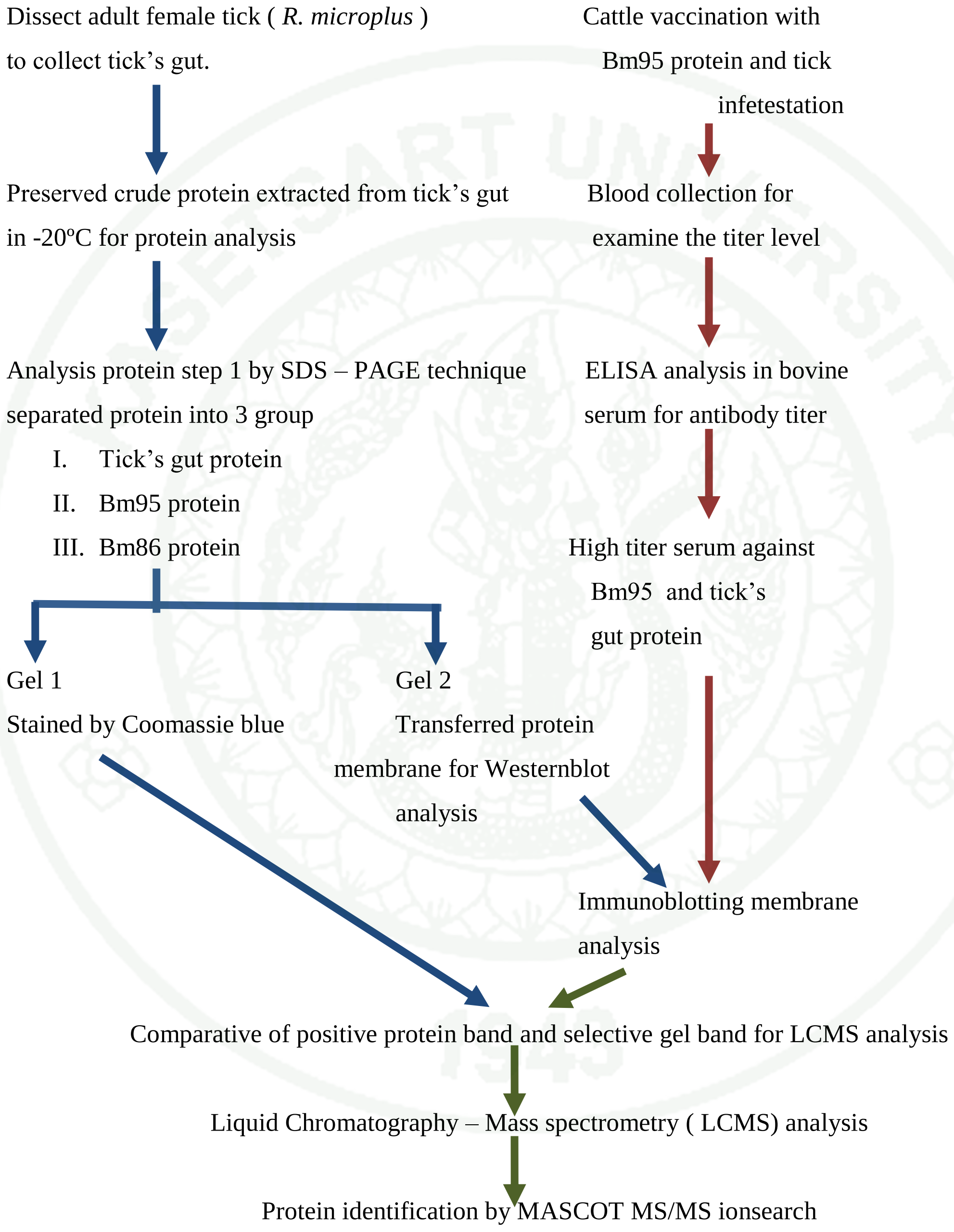


Figure 9 the flow chart indicated the detail of analyzed proteomic pattern of midgut proteins

1. Sample preparation

1.1 Tick gut protein preparation

R. microplus adult ticks in pre – engorged phase (size 3 – 4 mm) were fixed on paraffin, covered by the cold deionized water, and dissected to isolate the tick gut. Tick guts were preserved in lysis buffer (30mM Tris HCl, 7M Urea, 2M Thiourea, 4% CHAPS, pH 8.5). After homogenization and sonication, samples were precipitated by centrifugation at 16,000 g, 30 mins at 4ºC. The supernatant was kept by aliquots and preserved at - 20ºC until used.

1.2 Bm95 protein preparation

Bm95 was the glycoprotein that expressed in midgut epithelium of *R. microplus*. The protein expression method was described by Jittapalapong *et al* (2008). Total RNA of Bm95 were extracted from Thai *R. microplus* gut and amplified by RT – PCR product with Bm95 – specific primer. The specific PCR product was observed approximately 1,700 bp. Thai Bm95 gene was expressed by cloning in the pPICZαA vector for expressing in KM71H, and produced in the recombinant plasmid Bm95 (rBm95). rBm95 was used as candidate protein (positive control) for immunization vaccine for cattle to find the antibody titer level.

1.3 Bovine Bm95 high titer serum

Diary – cattle with 6 months old were injected by Bm95 recombinant protein vaccine (200 µg Bm95 soluble protein, 1 ml Montanide adjuvant) three times at three weeks intervals (Jittapalapong *et al*., 2008). After the last booster, animals were challenge infested by 3,000 larval tick and the blood collection was taken weekly. Sera were separated, and stored at -80ºC until analysis.

2. A method to detect antibody responses

Enzyme linked immunosorbent was used to determine antibody titer. Bm95 protein was coated in 96 well microplate with 100 µl/well which protein concentration in carbonate buffer (pH 9.6) was 5µg/ml and incubated the coated – microplate at 4ºC overnight. Afterwards, the plate was washed four times with phosphate buffer saline (PBS) 0.05% Tween 20, blocked non – coating space by 1% gelatin in carbonate buffer 3 hours at room temperature, and then washed four times. After that, 100 µl of serum (dilution 1:200) in PBS (pH 9.6) were added duplicated and incubated for 2 hours at room temperature, the plate was washed four times, then added 100 µl of secondary antibody peroxidase conjugated rabbit anti bovine IgG (A5295, Sigma – Aldrich) (dilution 1:5,000) incubated for 2 hours and wash four times. Finally, substrate (3,3',5,5' tetramethylbenzidine (TMB) (K blue® TMB substrate, Neogen Europe Ltd, Scotland, UK)) at 100 µl was added and waited for 5 min, then added 100 µl of 1N HCl to stop the reaction.

Optical density (O.D.) was measured by ELISA plate reader (Dynex Technologies®, Virginia, USA) at 450 nm. The highest titer serum was used as 1º antibody in immunoblotting analysis.

3. Sodium dodecylsulphate polyacrylamide gel electrophoresis

10% stacking gel of sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS – PAGE) was prepared and assembled in Mini – PROTEAN® 3 cell (Bio – Rad). Sample mixed with sample buffer (Laemmli sample buffer (Bio – Rad), and 5% β- mercaptoethanol) was heated at 90ºC for 5 min. Then, loaded sample and marker (Precision Plus Protein Standard, Kaleidoscope™, Bio – Rad) into gel lane. The sample was run in electrophoresis at 200 volts for 1 hour in 1% SDS Tris – glycine buffer. After the electrophoresis step, one gel was preserved in Transfer buffer (20% Methanol Tris – glycine buffer, pH 8.3) for western blot analysis, the other was transferred to fixative solution (40% methanol, 10% acetic acid) for 60 min and stained with colloidal Coomassie blue G – 250 (0.08% Coomassie brilliant blue G – 250, 1.6% Ortho – Phosphoric acid, 8% ammonium sulfate, 20% methanol) overnight.

Gel was washed two times in deionized water to remove background and conserved in deionized water until process to LC – MS analysis.

4. Immunoblotting analysis

Protein were transfer from gel to PVDF membrane (0.2 µm pore size Immobilon^{TM} – PSQ, Millipore Corporation, U.S.A.) by 100 volts, 100 min in Mini Trans – Blot$^{®}$ Electrophoretic Transfer Cell (Bio – Rad). Membrane was washed in washing buffer (TBS, pH 7.5, 0.005% Tween – 20) for 5 min and transferred to blocking buffer (TBS, pH 7.5, 3% gelatin, 0.5% Tween – 20) at room temperature for 3 hours. Dilution of serum (1: 100) in antibody buffer (TBS, pH7.5, 1% (w/v) gelatin, 0.5% Tween – 20) were added into incubated membrane for 3 hours. Membrane was washed and probed with 1:5000 dilution of peroxidase conjugate rabbit anti bovine IgG (A5295, Sigma – Aldrich) in antibody buffer for 1 hour. Washed the 2^{o} antibody and added DAB substrate (3,3' – Diaminobenzidine Enhanced Liquid Substrate System, Sigma) to generate color on membrane within 5 min.

5. Gel digestion of protein band

The gel partition were put through in – gel digestion step. The gel pieces were dehydrated with 100% acetonitrile (ACN), reduced with 10 mM DTT in 10 mM ammonium bicarbonate at room temperature for 1 hour in the dark with the combination solution of 100 mM iodoacetamide (IAA) in 10 mM ammonium biocarbonate. After alkylation process, the gel fractions were dehydrated twice with 100% ACN for 5 min. For performing in gel digestion of proteins, 10 µl of trypsin solution (10 ng/µl trypsin in 50% ACN/10 mM ammonium bicarbonate) was added to the gels and then incubated at room temperature for 20 min, and followed by add 20 µl of 30% ACN to keep the gels submerged throughout the digestion processing. The gel pieces were incubated at 37ºC for a few hour or overnight. For extraction of the peptide digestion products, 30 µl of 50% ACN in 0.1% formic acid (FA) was added into the gels, and followed by incubated the gel at room temperature for 10 min on the shaker machine. Peptide extracted products were collected and pooled together in the new micro-tube. The pool extracted peptides were dried by vacuum centrifuge and kept at -80ºC for further mass spectrometry analysis.

6. Liquid chromatography – mass spectrometry

The digested protein was injected into Ultimate 3000 LC System (Dionex, USA) coupled with ESI – Ion Trap MS (HCT Ultra PTM Discovery System (Bruker, Germany)) with electrospray at flow rate of 300 nl/min to a nanocolumn (Acclaim PepMap 100 C18, 3 µm, 100A, 75 µm 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 min.

7. Protein identification

MS data were interpreted to MASCOT generic – format (mgf) peak lists and sent to MASCOT MS/MS ionsearch (Matrix Science, London, UK (Perkin *et al*., 1999)). Selected NCBI as the database, other metazoa to taxonomy, trypsin was chosen as the digest enzyme and 1 missed cleavage was allowed. Carbamidomethyl at cysteine was used as a fixed modification with oxidation of methionine which was selected as variable modifications. Another parameters set for the search included peptide charge 1+ 2+ 3+, ESI – TRAP selected for the instrument type. In the MASCOT database search, Ion score is -10*log (P), where P is the probability that the observed match is a random event. Individual ion scores > 45 indicated identity or extensive homology ($p < 0.05$).

RESULT

SDS – PAGE and Western blot analysis

Form SDS – PAGE, the protein was separated in 10% stacking gel stained by Coomassie blue. Crude tick’s gut protein was separated into 17 bands which sized at 250, 180, 97, 80, 71, 68, 57, 50, 45, 37, 32, 30, 24, 22, 19, 17, and 16 kDa. Bm95 protein was consisted of 6 protein bands at 97, 80, 68, 57, 37 and 30 kDa. Finally, Bm86 was also consisted of 6 protein bands at 80, 68, 57, 41, 27, and 21 kDa. The diagramatic of protein bands form SDS – PAGE was demonstrated in Figure 10A. After comparative study of correlation proteins associated with antibody responses via Westernblotting membrane (Figure 10 B), the result showed 9 correlated proteins of tick’s gut protein within SDS – PAGE and blotting membrane at 97, 80, 68, 57, 45, 37, 30, 24, and 22 kDa. The correlated proteins from Coomassie stained gel and Westernblotting membrane of Bm95 protein demonstrated 6 bands which represented the same size as seem in SDS – PAGE gel. In addition, Bm86 analysis yielded 5 correlated proteins at 80, 68, 57, 41, and 21 kDa. From this result, the homologous proteins found in both SDS – PAGE gel and Westernblotting membrane in all samples (tick’s gut protein, Bm95, and Bm86) were 80, 68, and 57 kDa as shown in Figure 11.

For protein comparison, 11 homologous protein (3 pieces per protein size, a total of 33 gel pieces) associated with host Ab were extracted and put through the LCMS analysis. LCMS results were shown the protein at 97, 80, 68, 57, 45, 41, 37, 30, 27, 24, and 22 kDa. The diagram of all indentical protein bands was shown in Figure 12.

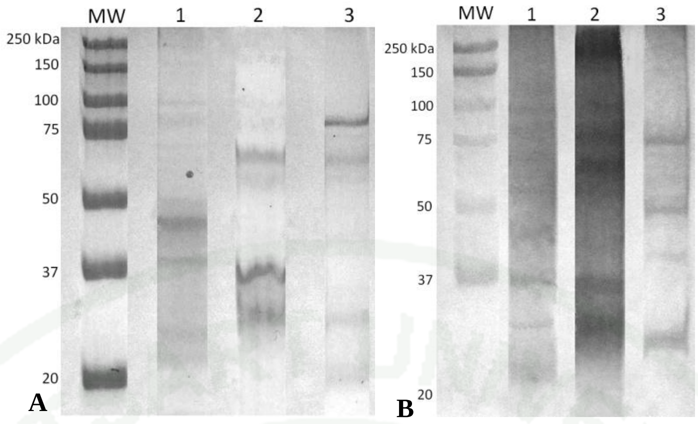


Figure 10 The presence of proteins found in gel and immunoblotting membranes. (**A**) Protein bands presented in SDS – PAGE with Coomassie blue staining. Mw: protein standard marker 250 kDa, L1: Crude tick's gut extraction protein, L2: Bm95 protein and L3: Bm86 protein. (**B**) The Western blot membrane showed the positive protein bands related with host Ab responses.

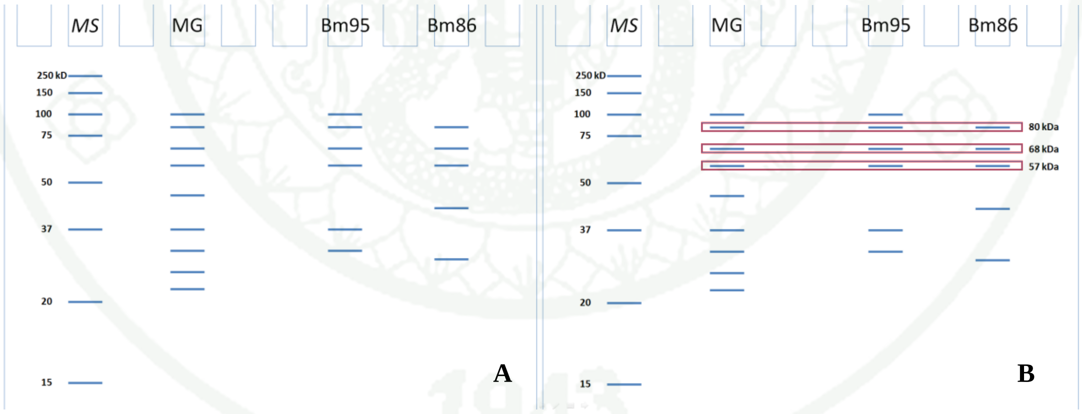


Figure 11 Diagrammatic picture shown the correlated MW bands between Coomassie gel and Western blot membrane. (**A**) By SDS – PAGE technique. MS: protein standard marker, MG: crude tick's gut protein, Bm95 protein, and Bm86 protein, respectively. (**B**) Selected protein groups associated with host immunity.

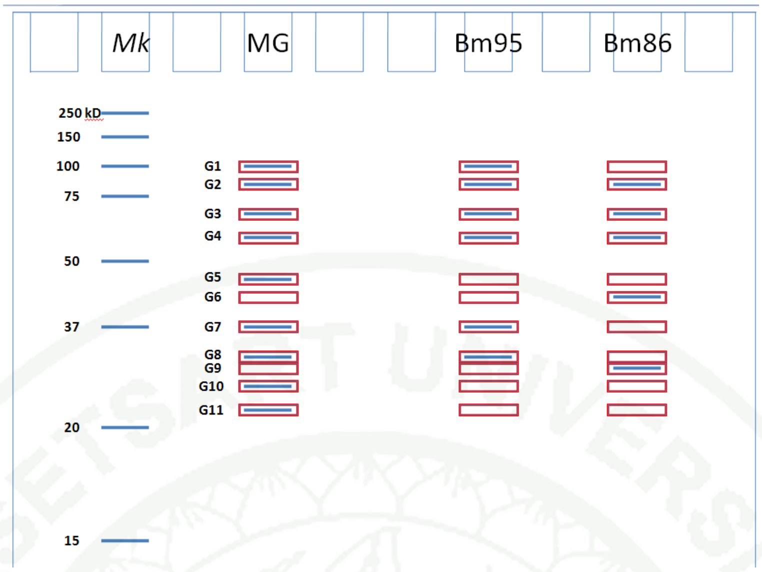
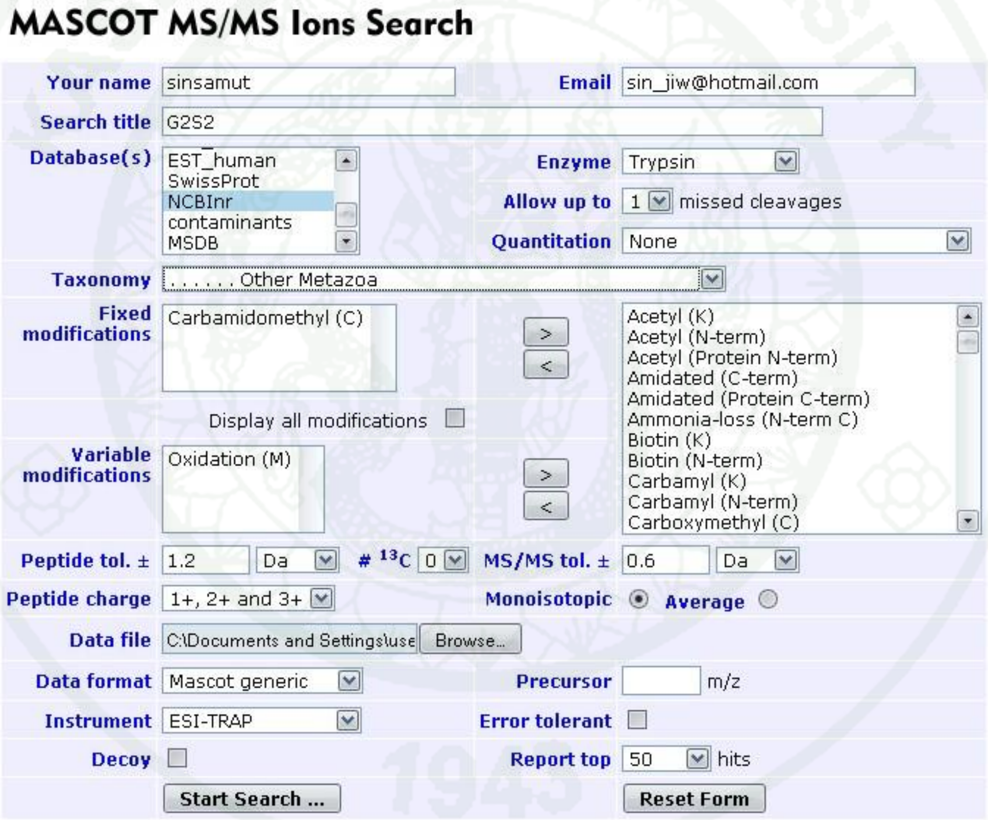


Figure 12 Diagrammatic picture showed the selective SDS – PAGE bands for LCMS analysis. The collected gel piece in each protein specimen was 11 samples (G1 – G11) and selected in the same protein size in each well.

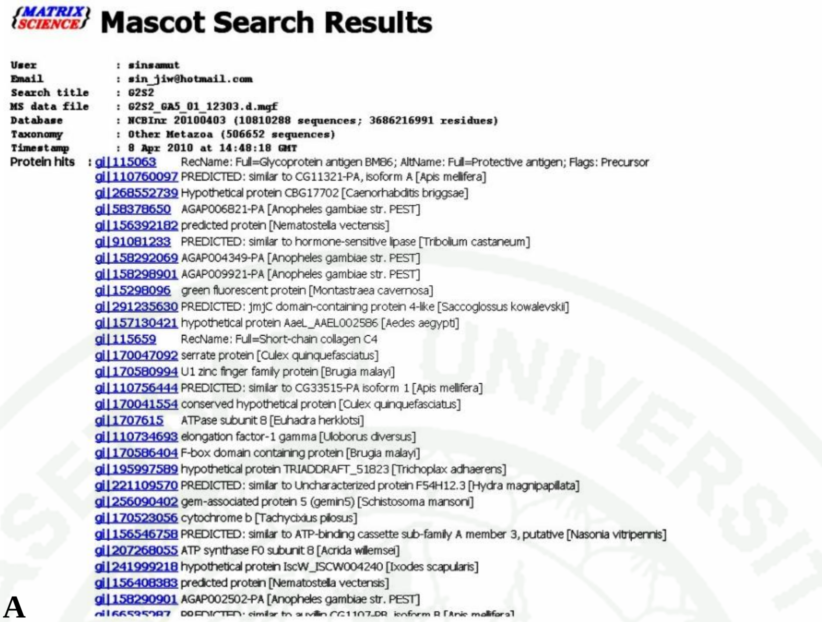
Liquid chromatography – Mass spectrometry (LCMS) analysis for protein indentification

The SDS PAGE gel pieces were digested, analyzed with LCMS and identified protein by Mascot (Matrix Science, London, UK (Perkin *et al.*, 1999) and reported the highest 50 protein as demonstrated in figure 13 - 14. Total gel pieces for LCMS analysis were 33 samples. The result data for LCMS analysis and Mascot search in each gel piece was shown in appendix A.


MASCOT MS/MS Ions Search

Your name: sinsamut
Email: sin_jiw@hotmail.com
Search title: G2S2
Database(s): NCBInr
Enzyme: Trypsin
Allow up to: 1 missed cleavages
Quantitation: None
Taxonomy: . . . . . . Other Metazoa
Fixed modifications: Carbamidomethyl (C)
Display all modifications ☐
Variable modifications: Oxidation (M)
Peptide tol. ± 1.2 Da
^{13}C 0
MS/MS tol. ± 0.6 Da
Peptide charge: 1+, 2+ and 3+
Monoisotopic ● Average ○
Data file: C:\Documents and Settings\use
Data format: Mascot generic
Precursor: m/z
Instrument: ESI-TRAP
Error tolerant ☐
Decoy ☐
Report top: 50 hits

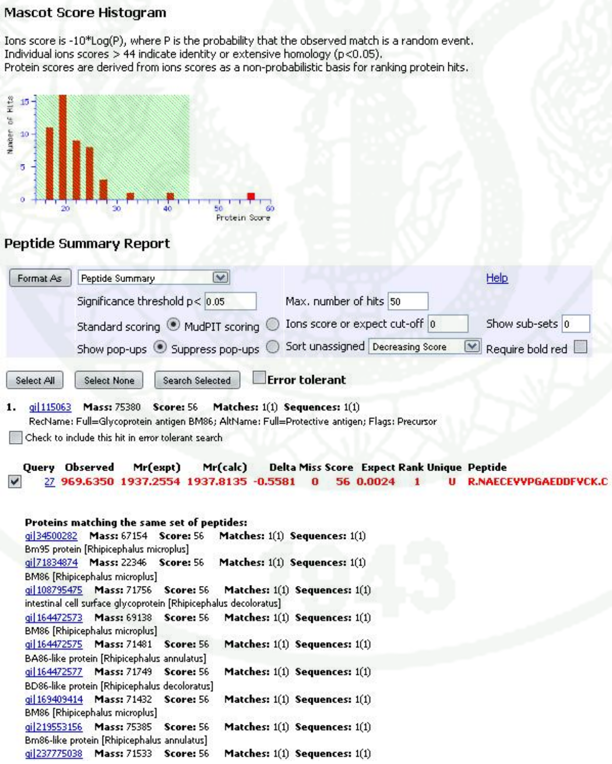
Figure 13 MS data was submitted to Mascot MS/MS ionsearch for protein identification



Mascot Search Results

User : sinsamut
Email : sin_jiw@hotmail.com
Search title : G2S2
MS data file : G2S2_GA5_01_12303.d.mgf
Database : NCBInr 20100403 (10810288 sequences; 3686216991 residues)
Taxonomy : Other Metazoa (506652 sequences)
Timestamp : 8 Apr 2010 at 14:48:18 GMT
Protein hits : gi|115063 RecName: Full=Glycoprotein antigen BM86; AltName: Full=Protective antigen; Flags: Precursor
gi|110760097 PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]
gi|268552739 Hypothetical protein CBG17702 [Caenorhabditis briggsae]
gi|58378650 AGAP006821-PA [Anopheles gambiae str. PEST]
gi|156392182 predicted protein [Nematostella vectensis]
gi|91081233 PREDICTED: similar to hormone-sensitive lipase [Tribolium castaneum]
gi|158292069 AGAP004349-PA [Anopheles gambiae str. PEST]
gi|158298901 AGAP009921-PA [Anopheles gambiae str. PEST]
gi|15298096 green fluorescent protein [Montastraea cavernosa]
gi|291235630 PREDICTED: jmjC domain-containing protein 4-like [Saccoglossus kowalevskii]
gi|157130421 hypothetical protein AaeL_AAEL002586 [Aedes aegypti]
gi|115659 RecName: Full=Short-chain collagen C4
gi|170047092 serrate protein [Culex quinquefasciatus]
gi|170580994 U1 zinc finger family protein [Brugia malayi]
gi|110756444 PREDICTED: similar to CG33515-PA isoform 1 [Apis mellifera]
gi|170041554 conserved hypothetical protein [Culex quinquefasciatus]
gi|1707615 ATPase subunit 8 [Euhadra herklotsi]
gi|110734693 elongation factor-1 gamma [Uloborus diversus]
gi|170586404 F-box domain containing protein [Brugia malayi]
gi|195997589 hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]
gi|221109570 PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]
gi|256090402 gem-associated protein 5 (gemin5) [Schistosoma mansoni]
gi|170523056 cytochrome b [Tachycixius pilosus]
gi|156546758 PREDICTED: similar to ATP-binding cassette sub-family A member 3, putative [Nasonia vitripennis]
gi|207268055 ATP synthase F0 subunit 8 [Acrida willemsei]
gi|241999218 hypothetical protein IscW_ISCW004240 [Ixodes scapularis]
gi|156408383 predicted protein [Nematostella vectensis]
gi|158290901 AGAP002502-PA [Anopheles gambiae str. PEST]

A



Mascot Score Histogram

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 44 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

Peptide Summary Report

Format As: Peptide Summary
Significance threshold p< 0.05
Max. number of hits 50
Standard scoring ● MudPIT scoring ○
Ions score or expect cut-off 0
Show sub-sets 0
Show pop-ups ● Suppress pop-ups ○
Sort unassigned Decreasing Score
Require bold red ☐

☐ Error tolerant

1. gi|115063 **Mass:** 75380 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
RecName: Full=Glycoprotein antigen BM86; AltName: Full=Protective antigen; Flags: Precursor
☐ Check to include this hit in error tolerant search

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
☑	27	969.6350	1937.2554	1937.8135	-0.5581	0	56	0.0024	1	U	R.NAECEVVPGAEDDFVCK.C

Proteins matching the same set of peptides:
gi|34500282 **Mass:** 67154 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
Bm95 protein [Rhipicephalus microplus]
gi|71834874 **Mass:** 22346 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
BM86 [Rhipicephalus microplus]
gi|108795475 **Mass:** 71756 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
intestinal cell surface glycoprotein [Rhipicephalus decoloratus]
gi|164472573 **Mass:** 69138 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
BM86 [Rhipicephalus microplus]
gi|164472575 **Mass:** 71481 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
BA86-like protein [Rhipicephalus annulatus]
gi|164472577 **Mass:** 71749 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
BD86-like protein [Rhipicephalus decoloratus]
gi|169409414 **Mass:** 71432 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
BM86 [Rhipicephalus microplus]
gi|219553156 **Mass:** 75385 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)
Bm86-like protein [Rhipicephalus annulatus]
gi|237775038 **Mass:** 71533 **Score:** 56 **Matches:** 1(1) **Sequences:** 1(1)

B

Figure 14 Mascot search results demonstrated data from Gel number 2 (G2;Bm95) protein searching. (**A**); result of accession number and protein name from G2 sample. (**B**); Mascot ionsearch presented the highest protein score form G2;Bm95 protein identification.

The Mascot database search indicated total 742 proteins within all gel band.The 7 unique peptides which individual ion score was extensive homology with ≥ 95% confidence (protein score > 44). The unique protein result was shown in midgut protein column including 1. hypothetical protein CBG18883 [*Caenorhabditis briggsae*](protein accession number is gi|268558454); 2. hypothetical protein TcasGA2_TC012552 [*Tribolium castaneum*] (gi|270013886); 3. PREDICTED: similar to predicted protein [*Tribolium castaneum*] (gi|91077092). Additionally, 2 proteins from 6 unique proteins appeared in Bm95 sample which were Bm95 protein [*Rhipicephalus microplus*] (gi|34500282), and RecName: Full=Short-chain collagen C4 (gi|115659). Bm86 sample presented two high score proteins namely angiotensin-converting enzyme-like protein [*Rhipicephalus microplus*] (gi|1468981), and Hypothetical protein CBG18883 [*Caenorhabditis briggsae*](gi|268558454) . The detail was demonstrated in table 4.

Table 4 Mascot database detail of MS/MS score identified with ≥ 95% confidence protein within individual protein column

Protein column	**Protein ID**	**Accesion number**	**Peptide sequence**	**protein score**	**MW**
Tick's gut column	Hypothetical protein CBG18883 [*Caenorhabditis briggsae*]	gi|268558454	R.CEIFNDVEKVETACIVER.I	47	302891
	hypothetical protein TcasGA2_TC012552 [*Tribolium castaneum*]	gi|270013886	R.MSSSGVSEMQFSMRSR.T + Oxidation (M)	45	92073
	PREDICTED: similar to predicted protein [*Tribolium castaneum*]	gi|91077092	R.KVQANIAR.K / R.LLPTNELPER.L	48	93691
Bm95 column	Bm95 protein [*Rhipicephalus microplus*]	gi|34500282	R.NAECEVVPGAEDDFVCK.C	72	67154

Table 4 (Continued)

Protein column	Protein ID	Accesion number	Peptide sequence	protein score	MW
Bm86 column	RecName: Full=Short-chain collagen C4	gi|115659	K.GDTGAPGPQGPK.G	47	36445
	angiotensin-converting enzyme-like protein [*Rhipicephalus microplus*]	gi|1468981	K.IAFLPFGYLLDK.W K.DKDLPLEPDLTR.N K.LSNEAASLDGYDNIK.S K.SAWLSDYETENMTEIVDK.L + Oxidation (M)	319	75495
	Hypothetical protein CBG18883 [*Caenorhabditis briggsae*]	gi|268558454	R.CEIFNDVEKVETACIVER.I	44	302891

The protein analysis indicated only 39 proteins (5.3 %, 39/742) were related to tick proteins as shown in table 5. These were yielded the attractive proteins such as Bm95 (gi|34500282), Bm86 – like protein (gi|164472575), glycoprotein Ag Bm 86 (gi|419953) which characterized as tick midgut epithelium glycoprotein used for vaccine against cattle ticks, angiotensin – converting enzyme (gi|1468981); the salivary kininased function as anti- inflammatory and vasodilation agent (Jarney *et al*., 1995), orcokinin (gi|186973149); the neuropeptide of abdominal ganglion and nerve fiber of gut (Li *et al*., 2002).

Another interesting protein was Cathepsin L – like cysteine proteinase (gi|94246073) which was proteolytic enzyme related to haemoglobin digestion. Additionally, the thioredoxin peroxidase (gi|33591156) which acts as growth factor, cytokine and chemotactic factor that secreted by antigen – presenting cells to stimulate T cell activation and T – cell growth (Nishinaka *et al*., 2002) were also indicated in this analysis.

Table 5 The 39 proteins related to tick protein and demonstrated form Mascot database.

Protein accession number	Protein Identificaiton	Molecular Weight	Peptide sequence
gi|34500282	Bm95 protein [*Rhipicephalus microplus*]	67154	R.NAECEVVPGAEDDFVCK.C K.ANCQCPPDTKPGEIGCIER.T
gi|1468981	angiotensin-converting enzyme-like protein [*Rhipicephalus microplus*]	75495	K.IAFLPFGYLLDK.W K.DKDLPLEPDLTR.N K.LSNEAASLDGYDNIK.S K.SAWLSDYETENMTEIVDK.L + Oxidation (M)
gi|164472575	BA86-like protein [*Rhipicephalus annulatus*]	71481	K.VLCECPWNQHLVGDK.C
gi|186973149	orcokinin precursor 1 [*Dermacentor variabilis*]	18254	R.LNGGELLR.S
gi|194246073	cathepsin L-like cysteine proteinase [*Dermacentor variabilis*]	16579	K.QNVGATDTGFVDIEQGSEDDLKK.A
gi|240953833	condensin-2 complex subunit H2, putative [*Ixodes scapularis*]	78660	R.EPYDIR.T
gi|241033935	hypothetical protein IscW_ISCW001706 [*Ixodes scapularis*]	81418	K.LLKVIVLTEPR.M
gi|241116846	ribonucleoside-diphosphate reductase large chain, putative [*Ixodes scapularis*]	43524	K.EFIHSIMLFLDNVLEDFINK.A + Oxidation (M)
gi|241117506	bone morphogenetic protein, putative [*Ixodes scapularis*]	47232	R.ILVMRFK.V
gi|241118202	hypothetical protein IscW_ISCW017486 [*Ixodes scapularis*]	10387	K.ERNPAK.G
gi|241146856	conserved hypothetical protein [*Ixodes scapularis*]	14220	R.CLEQNSWDLQK.A
gi|241153253	leucine rich repeats containing protein, putative [*Ixodes scapularis*]	22142	K.NYISDVETPR.L
gi|241155753	excitatory amino acid transporter, putative [*Ixodes scapularis*]	102695	K.DIVIRHVR.D

Table 5 (Continued)

Protein accession number	Protein Identificaiton	Molecular Weight	Peptide sequence
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [*Ixodes scapularis*]	56983	TLTHRVR.L
gi|241162965	hypothetical protein IscW_ISCW016627 [*Ixodes scapularis*]	9338	R.ERPFGAGR.E
gi|241186228	sdk-P1, putative [*Ixodes scapularis*]	191031	R.EGVPQAAPSGLR.A
gi|241293344	conserved hypothetical protein [*Ixodes scapularis*]	80487	R.EPNVVSQTAHSVARR.G
gi|241304593	hypothetical protein IscW_ISCW017719 [*Ixodes scapularis*]	35769	R.NQTGQAVCFR.C
gi|241351505	hypothetical protein IscW_ISCW004874 [*Ixodes scapularis*]	11366	R.EDPERSGR.G
gi|241557575	conserved hypothetical protein [*Ixodes scapularis*]	30620	R.HFHESAVGPR.Q
gi|241561644	synembryn, putative [*Ixodes scapularis*]	14334	K.LARGGVVQPAR.V
gi|241571336	hypothetical protein IscW_ISCW009045 [*Ixodes scapularis*]	25970	R.LDVLVLNFFI.-
gi|241594096	hypothetical protein IscW_ISCW009508 [*Ixodes scapularis*]	9092	-.MECLRSVLTIAGK.A
gi|241608961	hypothetical protein IscW_ISCW019269 [*Ixodes scapularis*]	15145	K.DGGLPPAVHFGAEARR.R
gi|241614959	hypothetical protein IscW_ISCW007319 [*Ixodes scapularis*]	59621	R.LLGPPEQMMGPQDQRMMGGPPDHR.M + 3 Oxidation (M)
gi|241618034	hypothetical protein IscW_ISCW010219 [*Ixodes scapularis*]	30351	R.ACGNLFKLDAFQK.L
gi|241654886	lipid exporter ABCA1, putative [*Ixodes scapularis*]	23912	K.AFPKTLSGGMK.Q
gi|241694276	conserved hypothetical protein [*Ixodes scapularis*]	34468	R.GGYLVKDLK.S
gi|241708208	LIM domain-binding protein, putative [*Ixodes scapularis*]	60818	K.IPICSNCGSPIR.G

Table 5 (Continued)

Protein accession number	Protein Identificaiton	Molecular Weight	Peptide sequence
gi|241713550	SMC protein, putative [*Ixodes scapularis*]	133226	-.MHIKSITIDGFK.S + Oxidation (M) K.QLEETKVNSDIQFTK.D
gi|241758359	protein wingless, putative [*Ixodes scapularis*]	36314	K.TCWKSLPAFSQVGAYLVQR.Y
gi|241788743	FGF receptor activating protein, putative [*Ixodes scapularis*]	30361	R.TGVALHTGPR.L
gi|241999176	neurofilament medium polypeptide, putative [*Ixodes scapularis*]	58842	K.QMPKPKKPK.K
gi|241999218	hypothetical protein IscW_ISCW004240 [*Ixodes scapularis*]	34876	R.RGTDNAAVFK.F
gi|241999928	kinesin-associated protein, putative [*Ixodes scapularis*]	77022	R.TPSGETASMAR.L + Oxidation (M)
gi|242002008	Niemann-Pick type C1 domain-containing protein, putative [*Ixodes scapularis*]	138159	R.QVPNLAPLPLDPR.K
gi|33591156	thioredoxin peroxidase [*Ixodes ricinus*]	19419	K.LTHPAPDFTGTAVVGGQFKDIK.L
gi|34597030	RNA polymerase II largest subunit [*Ballophilus australiae*]	10360	R.FQASQVQP.-
gi|419953	glycoprotein antigen BM86 - southern cattle tick (fragments)	21100	K.ANCQCPPDTRPGEIGCIEK.D

Furthermore, the result of proteomic analysis revealed the associated protein from other insect and nematode such as DNA - directed RNA polymerase III (gi|565 51159) which found in wasp and *Schistosoma mansoni* (gi|256086074). Beside this, the analysis yielded the unspecific function proteins for example, 206 hypothetical proteins, and 69 predicted proteins from *Nematostella vecensis* (gi|56349294), *Trichoplas adhaevens* (gi|95999698), and *Tribolium castaeum* (gi|91077092).

After comparison of proteins between tick's gut protein in Bm95 and Bm86 group, 80 homologous proteins from all 3 protein origins were found such as SCO – spondin (gi|115746571), member of the thrombospondin that inhibits angiogenesis (growth and repair of blood vessels)(Lawler, 2000), RNA polymerase III (gi|56551159) and elongation factor – 2 (gi|377039) which was related to RNA synthesis process, ATPsynthase FO subunit 8 (gi|207268055), the ATP synthesis and electron transport chain, angiotensisn – converting enzyme precursor (gi|2420211) and the tick salivary kininase.

Additionally, the data indicated 48 correlated proteins within tick's gut protein and Bm95. The interesting proteins were aminopeptide N (gi|70051303), a proteolytic enzyme related to the haemoglobin digesting process (Hatta *et al*., 2007), thioredoxin domain (gi|221130437), the T cell activator, Serpin (gi|240270422), a protein enable to inhibit blood clotting process, and imflammatory – complement activation (Armstrong *et al*., 2001).

For tick's gut protein's related to Bm86 analysis, 38 proteins were found between each group. Some major proteins such as cysteine protease (gi|289741131), the haemoglobin digestion enzyme, and myosin protein (gi|157106), the structural molecule of muscle were included (appendix Table B12).

The comparative analysis of protein in each gel at the same size revealed the shared protein profile between individual gel. As gel number 2, the proteomic analysis yielded 14 homologous proteins within tick's gut protein and Bm95, and 7 identical proteins within tick's gut protein and Bm86 group. More data was shown in Table 6.

Table 6 The homologous proteins between tick’s gut proteins, Bm95, and Bm86.

Gel number	**Number of homologus protein to Bm95**	**Number of homologus protein to Bm86**
G1	1	22
G2	14	7
G3	8	12
G4	4	4
G5	5	11
G6	12	10
G7	6	16
G8	8	2
G9	11	9
G10	11	6
G11	10	7

The analysis of SDS – gel staining with Coomassie blue and immunoblotting membrane demonstrated the related proteins at 80, 68, and 57 kDa form gel number 2, 3, 4, respectively. These related proteins indicated their immunogenicity among these proteins (tick’s gut, Bm95, and Bm86). After comparison of 3 these proteins, the result showed 22 resemblant proteins hits from gel piece number 2, 3 and 4 (Table 7). The analyzed result demonstrated the interesting proteins such as SCO – spondin, RNA polymerase III, and muscle myosin heavy chain (gi|193624646).

Table 7 All 22 resemblant proteins, presence in gel number 2, 3, and 4 in all 3 protein columns (tick's gut protein, Bm95, and Bm86).

Accesion number	**Protein identification**	**Peptide sequence**
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [*Apis mellifera*]	K.EPGQLQQLLR.D
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [*Apis mellifera*]	K.GVVGTIVVNGEGIPIK.S
gi|115623606	PREDICTED: hypothetical protein [*Strongylocentrotus purpuratus*]	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|115659	RecName: Full=Short-chain collagen C4	K.GDTGAPGPQGPK.G
gi|115746571	PREDICTED: similar to SCO-spondin [*Strongylocentrotus purpuratus*]	K.LETKGLFMSR.L
gi|156363128	predicted protein [*Nematostella vectensis*]	K.FPVRPLIIK.T
gi|156400082	predicted protein [*Nematostella vectensis*]	K.EDKLLDHR.D
gi|156541302	PREDICTED: similar to GA21542-PA [*Nasonia vitripennis*]	R.RVSEGNAPHSK.N
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [*Nasonia vitripennis*]	K.IINLAQGKPK.G
gi|157130421	hypothetical protein AaeL_AAEL002586 [*Aedes aegypti*]	K.SQKYGHVK.S
gi|158290901	AGAP002502-PA [*Anopheles gambiae* str. PEST]	K.MGPPMQGR.G + Oxidation (M)
gi|158292069	AGAP004349-PA [*Anopheles gambiae* str. PEST]	R.GNGGGGGGGGGSR.G
gi|170041554	conserved hypothetical protein [*Culex quinquefasciatus*]	M.ASLTIPKAPK.K
gi|170580994	U1 zinc finger family protein [*Brugia malayi*]	K.IDGPIEFR.K
gi|170586404	F-box domain containing protein [*Brugia malayi*]	R.QNALLIQPK.A
gi|1707615	ATPase subunit 8 [*Euhadra herklotsi*]	-.MPQLSPHSL.- + Oxidation (M)
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [*Acyrthosiphon pisum*]	K.DQVGQVNPPK.Y
gi|195997589	hypothetical protein TRIADDRAFT_51823 [*Trichoplax adhaerens*]	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [*Hydra magnipapillata*]	K.IKSNPIQPK.F
gi|242014857	hypothetical protein Phum_PHUM363220 [*Pediculus humanus corporis*]	K.ILKNLAGKPK.I
gi|268581039	Hypothetical protein CBG22754 [*Caenorhabditis briggsae*]	K.ENQKPIKPK.T
gi|270010424	hypothetical protein TcasGA2_TC009817 [*Tribolium castaneum*]	K.LMQLMVMK.H + 2 Oxidation (M)

From the protein correlation analysis, the data showed 188 unidentical proteins from tick’s gut, 194 proteins form Bm95, and 173 proteins from Bm86. These information suggested the important proteins of tick’s gut which might relate to host – parasite interaction such as serine protease inhibitor (serpin), an inhibitor of thrombin activity to delay blood coagulation time (Imamura *et al*., 2005), and Trialysisn, the salivary protein that can permabilize and lyse mammalian cell (Amino *et al*., 2002). For Bm95 group,the only specific protein found in this column such as Bm95, BA86 – like, and Bm86 protein which were tick midgut epithelium glycoprotein. The unidentical proteins presented in Bm86 column which related to host – parasite interaction were angiotensin converting enzyme like protein, tick salivary kininase, serpin, and cathepsin L.

DISCUSSION

From our experiment objective, we studied *R. microphilus*'s gut proteome to find out proteins associated with host immunity. This information was related to the selection of candidate proteins for improvement of vaccine against cattle's tick in the future. The previous tick protein studies have been initiated since 1978 and also associated with the properties for individual protein to develop an anti – tick vaccine to decrease the tick's population, tick's weight, and female fertility (Galun, 1978). The membrane – bound glycoprotein, Bm86 extracted and purified from the cattle tick have a property to produce the cattle immunity to prevent tick infestations (Willadsen *et al*., 1989; de la Fuente *et al*., 1998). Bm86 became the target protein and eventually become the commercial vaccine against cattle ticks as known as Gavac ™ and TickGARD® (Rodriguez *et al*., 1995 b). Additionally, Bm86 protein has been proven that the tick's gut protein is immunogenic and induces host immunity against tick infestations. Other proteins for vaccine such as Bm95, serpin, and angiotensin – converting enzyme (Bm91) were identified (Garcia – Garcia *et al*., 2000). In 2004, the previous study of proteome from unfed larval stage of cattle tick (*R. microplus*) were reported for 20 proteins significantly to peptide mass matching on NCBI database and most proteins was cuticle proteins from exoskeletal structure. In addition, the interesting proteins was glycine - rich immunodominat saliva protein and belong to the component of serpines family protein (Untalan *et al*., 2005). The proteomic analysis of midgut collected from fed female tick, *R. microplus* was done by a combination of 2DE gel electrophoresis and LCMS (Kongsutwan *et al*., 2010).

By correlated these two studies and our study, the difference of result was due to the different software database used to identify protein from two previous studies. From Kongsuwan *et al* (2010) and Untalan *et al* (2004), the database for protein recognition was done by EST database (cDNA sequence database) to identify gel spots form 2DE gel. In our experiment, we analyzed and identified the proteins associated with Ab response to target as candidate protein. All protein in our study was identified by MASCOT database. The protein analysis yielded the matching proteins correlated with previous study such as cysteine protease, aminopeptidase, cathepsin L – like cysteine proteinase (a proteolytic enzyme related to haemoglobin digestion reaction), actin, tubulin (the cytoskeletal structure), heat shock protein (the chaperone protein that activated during cell stress), thioredoxin (stimulated T cell activation and elongation factor, histone, riboneucloprotein) and 60s ribosomal protein (function in transcription – translation process).

To our study, the tick's gut proteomic analysis of *R. microplus* demonstrated proteins associated with host Ab response which might be the candidate protein to use as the part of combined protein with Bm95 or Bm86 in the multivalent vaccine in the future. These proteins were exemplified by sereine protease inhibitor (serpins), SCO – spondin protein, and angiotensin – converting enzyme. Serine protease inhibitor (serpins) is a common protein finding in the saliva of amongst haematophagous parasites. This protein effected by inhibits blood coagulation enzyme, particularly thrombin in competitive substrate inhibition mechanism (Naski *et al*., 1990). Furthermore, the salivary gland can synthesize serpins protein from DNA demonstrated by the study of *Haemaphysalis longicornis* (Sugino *et al*., 2003). The effect of serpin vaccine was shown by the increased mortality of *H. longicornis* (Imamura *et al*., 2005). The other protein was SCO – spondin protein which was the thrombospondin family and was functioned in stimulation or inhibiting the proliferation and migration of vascular smooth muscle or endothelial cell in the row of repair and generate blood vessel (Lawler, 2000). The last proteion was angiotensin – converting enzyme which was a tick kininase. This protein was expressed within the gut and salivary gland (Jarney *et al*., 1995) and had a role by increasing the efficacy of the Bm86 vaccine when used by co – administered (Willadsen *et al*., 1996).

Beside this, our experiment demonstrated several proteolytic enzymes in haemoglobin digestion reaction which will be the perspective proteins for vaccine targets. These proteins were characterized by inhibiting the digestion of blood meal in parasite by suppressing cytosolic gut peptidase led to the reduction of tick *H. longicornis* fertility (Hatta *et al*., 2007).We also identified the proteins from the member of proteolytic enzyme such as cathepsin L – like cysteine proteinases, OTU – like cystein protease, and aminopeptidase N protein.

The identification of each protein depended on protein database such as NCBI, Swiss Prot or specific database for specific organism for instance EST_human, and EST_mouse. However, the tick's protein is still limited so that this will affect the investigation of novel tick proteins. This effect was characterized when compared the tick's protein within the database, but the software compared the peptide sequence with another resemblant peptide sequences which expressed from the different organisms such as insect or nematode. Due to this reason, our study demonstrated only 5% (39/742) from tick proteins. Likewise, the previous experiment had demonstrated the correlated proteins within other insect and nematode for example; *Anopheles gambiae*, *Mesobuthus cyptius*, *C. elegans*, and *Tachypleus tridentatus* (Xu *et al*., 2005).

Nevertheless, even if our result showed the presence of the recombinant Bm95 protein from Bm95 column, but the analysis could not recognize Bm86 protein from recombinant Bm86 protein column. The reason of this was due to inaccurate expression of recombinant Bm86 protein system. In addition, the result presented Angiotensin – converting enzyme – like protein (ACE) with individual MS/MS scores identified with ≥ 95% confidence, indicating identity or extensive homology in Bm86 sample. Using the analysis of peptide sequences by EMBOSS align software, the result yielded 0.7% identity and 0.9% similarity between Bm86 and ACE protein peptides. This information indicated that bot protein do not have the homolog peptide sequences. The comparison of DNA sequence Bm86 and ACE were performed by using open – reading frame programe (BCM search engine). The result showed that the deduce amino acid of Bm86 and ACE were not homologous since Bm86 expression was processed in *E. coli* system instead of yeast system. Some gycosylation in these proteins might be missing. The other possibility was the prsence of other homolog peptides sequence from *E. coli* might be contaminated into the Bm86 protein. However, after the analysis of MS data in *E. coli* taxonomy, the information shown no peptide sequences of ACE with *E. coli* peptides. Therefore, the

ACE peptide sequence might be related to tick’s protein sequences more than *E. coli* sequences.

The conclusion of unidentified protein Bm86 from and the presence of ACE in Bm86 were not clearly understood. The possible reason suggested that there was the error in the DNA cloning process, the non – specific amplification by primer for Bm86 DNA sequence and the false protein construction form *E. coli* cell. This will be the evidence to be considered for future study of tick protein analysis.

From our analysis, the preliminary information about protein related to host immunity was shown. For more specific details of protein profiles, the further study should be more completely design and used specific, completely database by using proteomic program to analyse the protein quantitative in each sample and to compare the colorized antigen – antibody reaction. Moreover, the complete tick’s protein database will be enhancing the study of tick’s proteins, and fulfil the more complete tick protein identification.

LITERATURE CITED

Aebersold, R. and M. Mann. 2003. Mass spectrometry – based proteomics. **Nature**. 422:198 – 207

Agbede, R.I.S. and D.H Kemp. 1986**.** Immunisation of cattle against *Boophilus microplus* using extracts from adult female ticks: histopathology of ticks feeding on vaccination cattle. **Int. J. Parasitol**. 16: 35-41

Agyei, A.D., and N.W. Runham. 1995. Studies on the morphological changes in the midguts of two Ixodid tick species *Boophilus microplus* and *Rhipicephalus appenduculatus* during digestion of the blood meal. **Int.J.Parasitol**. 1:55 – 62

Akhtar, M., C.S. Hayat and Z. Iqbal, 1999. Antigenic response of midgut (*Boophilus microplus*) cell culture vaccine. **Small Ruminant Research**. 33:189 – 192

______, ______, M. Ashfaque, Z. Iqbal and M. Afag. 1992. In vitro propagation of midgut cell of *Boophilus microplus*. **Pakistan Vet.J**. 12:196

Amino, R., R.M. Martins, J. Procopio, I.Y. Hirata, M.A. Juliano and S. Schenkman. 2002. Trialysis, a novel pore – forming protein form saliva of hematophagous insects activated by limited proteolysis. **J.Biol.Chem**. 277:6207 – 6213

Andreotti, R., A. Gomes, C.A.M. Sampaio and A.S. Tanaka. 2000. Immunoprotection induced in calves by serine proteinase inhibitor from *Boophilus microplus* unfed larvae. **Rev.Bras.Parasitol.Vet**. 9:58 – 89

Armstrong, P.M., L.R. Brunet, A. Spielmen, and S.R. Telford IIIrd. 2001. Risk of lyme disease: perceptions of residents of a lone star tick – infested community. **Bull.World.Health.Organ**. 79:916 – 925

Boué, O., M. Redondo, C. Montero, M. Rodríquez and J. de al Fuente. 1999. Reproductive and safety assessment of vaccination with GAVAC^{TM} against the cattle tick (*Boophilus microplus*). **Theriogenology**. 51:1547 – 1554

Careri, M., A. Mangia and M. Musci. 1996. Application of liquid chromatography – mass spectrometry interfacing systems in food analysis: pesticide, drug, and toxic substance residues. **J.Chromatography.A**. 727:153 – 184

Carr, S.A., M.E. Hemling, M.F. Bean and G.D. Robert. 1991. Integration of mass spectrometry: a novel method for identifying attachment sites of Asn – linked sugar in glycoproteins. **Anal.Chem**. 63:2802 – 2824

Casadevall, A. and L. Pirofski. 2003. Antibody – mediated regulation of cellular immunity and the inflammatory response. **Trend in Immunology**. 24:474 – 478

Cobon, G.S. and P.Willadsen. 1990. Vaccines to prevent cattle tick infestations. **In *New Generation Vaccines*** (ed. Woodraw, G.C., and Levine, M.M.). Marcel.Dekker Inc.New York.901 – 917

Cooper, M. 1977. The life cycle of ticks: In **Cattle tick Control**. Copper McDougall& Robertson Ltd. Berkhamsted England. 10

Corthals, G.L., V.C. Wasinger, D.F. Hochstrasser and J.C. Sanchez. 2000. The dynamic range of protein expression: a challenge for proteomic research. **Electrophoresis**. 21:1104 – 1115

Covey, T.R., R.F. Bonner, B.I. Shushan and J. Henion. The determination of protein, oligonucleotide and peptide molecular weights by ion – spray mass spectrometry. **Rapid.Commun.mass.Spectrom**. 2:249 – 255 de la Fuente, J. and K.M. Kocan. 2003. Advances in the identification and characterization of protective antigens for development of recombinant vaccines against tick infestations. **Expert.Rev.Vaccines**. 2:583 – 593

______, C. Almazán, U. Blas- Machado, V. Naranfo, A.J. Mangold, E.F. Blouin, C. Gortazar and K.M. Kocan. 2006(a). The tick protective antigen, 4D8, is a conserved protein involved in modulation of tick blood ingestion and reproduction. **Vaccine.** 24:4082 – 4065

______, M. Canales and K.M. Kocan. 2006(b). The importance of protein glycosylation in development of novel tick vaccine strategies. **Parasite.Immunol**. 28:687 – 688

______, M. Rodriguez and J.C. Garcia – Garcia. 2000. Immunological control of ticks through vaccination with *Boophilus microplus* gut antigen. **Ann.N.Y.Acad.Sci**. 916:617 – 621

______, ______, M. Redondo, C. Montero, , J.C. Garcia – Garcia, L. Mendez, E. Serrano, M. Valdes, A. Eneiquez, M. Canales, E. Ranos, O. Boué, H. Machado, R. Lleonart, C.A. de Armas, S. Rey, J.L. Rodriquez, M. Artiles and L. Garcia. 1998. Field studies and cost – effectiveness analysis of vaccination with GAVAC^{TM} against the cattle tick *Boophilus microplus*. **Vaccine**. 16:366 – 373

Dunn, M.J. 2000. From Genome to Proteome: **Advances in the Practice and Application of Proteomics**. Wiley – VCH, New York.

Fawcett, D.W., K.C. Bennington and T.R. Voight. 1986. The cell biology of the ixodid tick salivary gland. *In*: Sauer, J.R., T.R. Hair. (Eds.), **Morphology, Physiology and Biology of Ticks**. Ellis Howood.Chichester. 22 – 45

Fenn, J.b., M. Mann, C.K. Meng, S.F. Wong and C.M. Whitehouse. 1990. Electrospray ionization for mass spectrometry of large biomolecules. **Science**. 246:64 – 71

Galun, R. 1978. Research into alternative arthropod control measure against livestock pests (Part I). **In: Work, Ecology and Control of External Parasite of Economic Importance on Bovines in Latin America.** CIAT, Columbia. 123 -130

Garcia – Garcia, J.C., C. Montero, M. Redondo, M. Vargas, M. Canals, O. Boue, M. Rodriguez, M. Joglar, H. Machado, I.L. González, M. Valdéz, L. Méndez and J. de la Fuente. 2000. Control of ticks resistant to immunization with Bm86 in cattle vaccinated with the recombinant antigen Bm95 isolated from the cattle tick, *Boophilus microplus*. **Vaccine**. 18:2275 – 2287

______, I.L. Gonzalez, D.M. Gonzalez, M. Valdéz, , L. Mendez, , J. Lamberti, , B. D’ Algostino, D. Citroni, H. Fragoso, M. Ortiz, M. Rodríquez, and F. de la Fuente. 1999. Sequence variation in the *B. micorplus* Bm86 locus and implications for immunoprotein in cattle vaccinated with this antigen. **Exp.Appl.Acarol**. 11:883 – 895

Gauss, C., M. Kalkum, M. Lowe, H. Lehrach and J. Klose. 1999. Analysis of the mouse proteome. (I) Brain proteins: separation by two – dimensional electrophoresis and identification by mass spectrometry and genetic variation. **Electrophoresis**. 20:575 – 600

Gindin, G., M. Samish, G. Zangi, A. Mishoutchenko and I. Glazer. 2002. The susceptibility of different species and stages of tick to enctomopathogenic fungi. **Exp.Appl.Acarol**. 28:283 – 288

Gorg, A., C. Obermaier, G. Boquth, A. Harder, B. Scheibe, R. Wildqruber and W. Weiss. 2000. The current state of two – dimensional electrophoresis with immobilized pH gradients. **Electrophoresis**. 21:1037 – 1053

Grandjean, O. 1984. Blood digestion in Ornithodorus moubata females.I. Biochemical changes in the midgut lumen and ultrastructure of the midgut cell, related to intracellular digestion. **Acarologia**. 25:147 – 165

Gygi, S.P., G.L. Corthais, Y. Zhang, Y. Rochon and R. Aebersold. 2000. Evaluation of two – dimensional gel electrophoresis – based proteom analysis techonology. **Proc.Natl.Acad.Sci.USA**. 97:9390 – 9395

______, Y. Rochon, B.R. Franza and R. Aebersold. 1999. Correlation between protein and mRNA abundance in yeast. Mol.Cell.Biol. 19:1720 – 1730

Hatta, T., R. Umemiya, M. Liao, H. Gong, T. Harnnoi, M. Tanaka, T. Miyoshi, D. Boldbaatar, B. Battsetseg, J. Zhou, X. Xuan, N. Tsuj, D. Tayler and K. Fujisaki. 2007. RNA interference of cytosolic leucine aminopeptidase reduces fecundity in the hard tick, *Haemaphysalis longicornis*. **Parasitol.Res**. 100:847 – 854

Herbert, B. 1999. Advance in protein solubilisation for two – dimensional electrophoresis. **Electrophoresis**. 20:660 – 663

Horn, S. 1987. Ectoparasites of animals and their impact on the economy of South America. **In 23rd World Veterinary Congress**. Montreal Proceeding.

Imamura, S., S. Vas.Junior Ida, M. Sugino, K. Ohashi and M. Onuma. 2005. A serine proteinase inhibitor (serpin) from *Haemaphysalis longicornis* as an anti – tick vaccine. **Vaccine**. 23:1301 – 1311

Institute for International Cooperation in Animal biologics, Iowa State University. 2007. *Rhipicephalus (Boophilus) microplus*. **Souther Cattle Tick, Cattle Tick**. Available Source: http://www.bcfsph.iastate.edu/Factsheets/pdfs/ boophilus_ microplus.pdf, January 20, 2008.

Jackson, L.A. and J.P. Opdebeeck. 1990. Humoral immune response of Hereford cattle vaccinated with midgut antigen of the cattle tick *Boophilus microplus*. **Parasite Immunol**. 12: 141

Jarney, J.M., G.A. Riding, R.D. Person, R.V. Mckenna and P. Willadsen. 1995. Corboxypeptidase from *Boophilus microplus*: a “Concealed” antigen with similarity to angiotensin – converting enzyme. **Insect. Biochem.Mole.Bio.** 25:969 – 974

Jaworski, D.C. 2003. Tick “talk”: protein release by tick salivary cells. **Trend Parasitol**. 19:427 – 429

Jittapalapong, S., S. Thanasilp, C. Kengradomkit, T. Sirinarukmit, G. Kaewmongkol and R.W. Stich. 2008. Molecular cloning, sequence analysis, and immune recognition of Bm95 from Thai strains of *Rhipicephalus* (*Boophilus*) *microplus*. **Ann.N.Y.Acad.Sci**. 1149:45 – 48

Johnson, L.A., D.H. Kemp and R.D. Pearson. 1986. Immunization of cattle against *Boophilus microplus* using extracts derived from adult female ticks: effect of induced immunity on tick population**. Int.J.Parasitol**. 16:27 – 34

Jonsson, N.N. 1997. Control of cattle tick (*Boophilus microplus*) on Queensland dairy farms. **Aus.Vet.J**. 75:802 – 807

Karas, M. and F. Hillenkamp. 1988. Laser desorption ionization of protein with molecular masses exceeding 10000 daltons. **Anal.Chem**. 60:2299 – 2301

Kashino, S.S., J.S. Resende, A.M. Sacco, C. Rocha, C. Proenca, W.C. Carvalho, A.A. Firmino, R. Queiroz, M. Benavides, L.J. Gershiwin and K.F. de Santos. 2005. *Boophilus microplus*: The pattern of bovine immunoglobulin isotype response to high and low tick infestations. **Exp.Parasitol**. 111:12 – 21

Kemp, D.H., R.D. Pearson, J.M. Gough and P. Willadsen. 1989. Vaccination against Boophilus microplus: localization of antigens on tick gut cells and their interaction with the host immune system. **Exp.Appl.Acarol**. 7:43 – 58

Kongsuwan, K., P. Josh, Y. Zhu, R. Pearson, J. Gough and M.L. Colgrave. 2010. Exploring the midgut proteome of partially fed female cattle tick (*Rhipicephalus* (*Boophilus*) *microplus*). **J.Insect.Physio**. 56:212 – 226

Labarta, V., M. Rodríquez, M. Penichet, R. Lleonart, L.L. Luaces and J. de la Fuente. 1996. Simulation of control strategies for the cattle tick *Boophilus microplus* employing the vaccination with a recombinant Bm86 antigen preparation. **Vet.Parasitol**. 63:131 - 160

Lai, R., S. O'Brien, T. Maushouri, A. Rogers, H. Kantarjian, M. Keating and M. Albitar. 2002. Prognostic value of plasma interleuking- 6 levels in patients with chronic lymphocytic leukemia. **Cancer**. 95:1071 – 1075

Lawler, J. 2000. The functions of thrombospondin -1 and -2. **Cell.Biol**. 12:634 – 640

Lara, F.A., U. Lins, G. Paiva – Silva, I.C. Almeida, C.M. Braga, F.C. Miquens, , P.L.Oliveira and M. Densa – Petreski. 2003. A new intracellular pathway of heam detoxification in the midgut of the cattle tick *Boophilus microplus*: aggregation inside a specialized organell, the hemosome. **J.Exp.Biol**. 206:1707 – 1715

Li, L., S.R. Pulver, W.P. Kelly, V. Thirumalai, J.V. Sweedler and E. Mander. 2002. Orcokinin peptides in developing and adult crustacean stomatogastric nervous systems and pericardial organs.**J.Comparative.Neuro**. 444:227 – 244

Lodos, J., O. Boué and J. de la Fuente. 2000. A model to simulate the effect of vaccination against *Boophilus* ticks on cattle. **Vet.Parasitol**. 87:315 – 326

Mann, M. 1990. Electrospray: It's potential and and limitation as an ionization method for biomolecule. **Org.Mass.Spectrum**. 25:575 – 587

Meng, C.K., M. Mann and J.B. Fenn. 1988. Electrospray ionization of some polypeptide and small protein. **Presented at the 36th ASMS conference on Mass Spectrometry and Allied Topics**. San Francisco, CA. 5 – 10 June 1988.

Mulenga, A., C. Sugimoto and M. Onuma. 2000. Issues in tick vaccine development: identification and characterization of potential candidate vaccine antigens. **Microbes Infect**. 2:1353 – 1361

Naski, M.C., J.W. Fenton II, J.M. Maragonore, S.T. Olson and J.A. Shafer. 1990. The COOH – terminal domain of hirudin. **J.Biol.Chem**. 265:13484 – 13489

Niessen, W.M.A. 1999. State o f the art in liquid chromatography – mass spectrometry.**J.Chromatogr**. 856:179 – 197

______. and A.P. Tinke. 1995. Liquid chromatography – mass spectrometry general proiciple and instrumention. **J.Chromatogr**. 703:37 – 57

Nishinaka, Y., K. Nakamura and J. Yodoi. 2002. Thioredoxin cyctokine action. **Methods.Enzymol**. 347:332 – 338

Nolan, J. and H.J. Schnitzering. 1986. Drug resistance in arthropod parasites. **In Chemotherapy of Parasitic Disease**. (ed. Campell, W.C., and Rew, R.W.). Plenum Pub. Corp. 603 – 620

______, J.T. Wilson, P.E. Green and P.E. Bird. 1989. Synthetic pyrethroid resistance infield samples of the cattle tick (*Boophilus microplus*). **Aus.Vet.J**. 66:179 – 182

Nunes, E.T., G.H. Bechara, K.C. Saito, S.E. Denardi, P.K. Oliveira and M.I.C. Mathias. 2005. Morphological, histological, and ultrastuctural characterization of degenerating salivary glands in female of the cattle – tick *Rhipicephalus* (*Boophilus*) *microplus*. (CANESTRINI, 1887) (Acari: Ixodidae). **Micron.** 36:437 – 447

Nuttall, P.A., A.R. Trimnell, M. Kazimirova and M. Labada. 2006. Exposed and concealed antigen as vaccine targets for controlling ticks'and tick – borne diseases. **Parasite. Immunol**. 28:155 – 163

O'Farell, P.H. 1975. High resolution two – dimensional electrophoresis of proteins. **J. Biol. Chem**. 250:40007 – 4021

Opdebeeck, J.P., J.Y.M. Wong, L.A. Jackson and C. Dobson. 1988(a). Vaccine to protect Hereford cattle against the cattle tick, *Boophilus microplus*. **Immunology**. 63:363

______, ______, ______ and ______. 1988(b). Hereford cattle immunized and protected against *Boophilus microplus* with soluble and membrane associated antigens from the midgut of ticks. **Parasite Immunol**. 10:405

______, ______ and C. Dobson. 1989. Hereford cattle protected against *Boophilus microplus* with antigens purified by immunoaffinity chromatography from larval and adult ticks. **Immunol**. 67:388 – 393

Patterson, S.D. and R.H. Aebersold. 2003. Proteomics: the first decade and beyond. **Nature.Gene.Supple**. 33:311 – 323

Perkins, D.N., D.J.C. Pappin, D.M. Creasy and J.S. Cottrell. 1999. Probability – based protein identification by searching sequence database using mass spectrometry data. **Electrophoresis**. 20:3551 – 3567

Petricoin, E.F., K.C. Zoon, E.C. Kohn, F.C. Barret and L.A. Loitta. 2002. Clinical proteomics: translating benchside promise into beside reality. **Nature.Rev.Drug Discv**. 1:683 – 695

Pevsner, J. 2003. Chapter8: Protein Analysis and Proteomics; **Bioinformatics and Functional Genomics**. A John Wiley & Son, Inc, Publication. 248 – 249

Rabilloud, T. 2002. Two – dimensional gel electrophoresis in proteomics: old, old fashioned but it still climbs up the mountains. **Proteomics**. 2:3 – 10

______, J.M. Strub, S. Luche, A. van Dorsselaer and J. Lunard. 2001. A comparison between Sypro Ruby and ruthenium II tris (bathophinanthroline disulfonate) as fluorescent stains for protein detection in gels. **Proteomics**. 1:699 – 704

Rand, K.N., T. Moore, A. Sriskantha, K. Spring, R. Tellam, P. Willadsen and G.S. Cobon. 1989. Cloning and expression of a protective antigen from the cattle tick *Boophilus microplus*. **Proc.Natl.Acad.Sci**. 86:9657 – 9661

Redondo, M., H. Fragoso, M. Ortíz, C. Montero, J. Lana, J.A. Medellin, R. Fría, , V. Hernández, R. Franco, H. Machado, M. Rodríquez and J. de al Fuente. 1999. Intergrated control of acaricide – resistant *Boophilus microplus* population on grazing cattle in Mexico using vaccination with GAVAC^{TM} and amidine treatment. **Exper.App.Acarol**. 23:841 – 849

Ribeiro, J.M.C. 1995. How ticks make a living. **Parasitology Today**. 11:91 – 93

Rodríquez, M., C.L. Massarol, A.H. de Fonseca, N.F. Ramos, H. Machado, V. Labarta and J. de al Fuente, 1995(c). Effect of vaccination with a recombinant Bm86 antigen preparation on natural infestation of *Boophilus microplus* in grazing dairy and beef pure and cross – breed cattle in Brazil. **Vaccine**. 13:1804- 1808

______, ______, V. Labarta and J. de la Fuente. 1995(b). Effect of vaccination with GAVAC^{TM} on the incidence of *Babesia bovis* infection and the reduction in the number and frequency of acaricide treatments in cattle under production conditions in Cuba. **Recombinants Vaccines for the Control of Cattle Tick**. Elfos Scientai, Havana,Cuba. 187 – 194

______, M.L. Penichet, A.E. Mouris, V. Labarta, L.L. Luaces, R. Rubiera, C. Cordovés, P.A. Sánchez, E. Ramos, A. Soto, M. Canales, D. Palenzuela, A. Triquero, R. Lleonart, L. Herrera and J. de la Fuente. 1995(a). Control of *Boophilus microplus* populations in grazing cattle vaccinated with a recombinant Bm86 antigen preparation. **Vet.Parasitol**. 57:339 – 349

______, R. Rubiera, M.L. Penichet, R. Montesinos, J. Cremata, V. Falcon, , G. Sanchez, R. Bringaz, C. Cordovés, M. Valdes, R. Lleonart, L. Herrera, and J. de la Fuente. 1994. High level expression of the *B. microplus* Bm86 antigen in the yeast *P. pastoris* forming highly immunogenic particles for cattle. **J.Biotech**. 33:135 – 156

Saito, K.C., G.H. Bechara, É.K. Nunes, P.R. de Oliveira, S.E. Denardi and M.I.C. Mathias. 2005. Morphological, histological, and ultrastructural studies of the ovary of the cattle – tick *Boophilus microplus* (Canestrini, 1887)(Acari: Ixodidea). **Vet.Parasitol**. 129:299 – 311

Sambrook, J. and D.W. Russell, 2001. Appendix9: Detection System; **Molecular Clonning a Laboratory Manual 3rd edition**. Spring Gabor Labolatory Press. New York. A9.28

Sauer, J.R., J.L. McSwain, A.S. Bowman and R.C. Essenberg. 1995. Tick salivary gland physiology. **Ann.Rev.Entomol**. 40:245 – 267

Schnitzerling, H.J., J. Nolan and S. Hughes. 1989. Toxicology and metabolism of isomers of flumethrin in larvae of susceptible and resistant strain of the cattle tick *Boophilus microplus* (Can,) (Acari: Ixodidae). **Exp.Appl.Acarol**. 6:47 – 54

Schumaker, T.T. and N.M. Serra – Freire. 1991. Histologia dasglâdulas salivares de adultos de Argas (Persicargas) miniatus (Koch, 1844) (Ixodidea: Argasidae) em jejum em alimentação e alimentados. **Rev.Bras.de Entamol**. 35:49 – 72

Seedbeck, R.M., P.H. Springell and J.C. O’Kelly. 1971. Alterations in host metabolism by the specifics and anoretic effect of the cattle tick (*Boophilus microplus*): I. Food intake and bodyweight growth. **Aus.J.Biol.Sci**. 24:373 – 380

Serrano, E., L. Méndez, M. Valdés and G.Seoane. 1995. Diagnosis and epizootiological situation of tick – borne cattle disease in Cuba. In: de la Fuente J (ed), Recombinant vaccines for control of cattle tick. **La. Habana:Elfos. Scientiae**. 27 – 36

Smith, R.D., J.A. Loo, C.G. Edmond, C.L. Barinaga and H.R. Udseth. 1990. New development in biochemical mass spectrometry: Electrospray ionization. **Anal.Chem**. 62:882 – 899

Smith, R.D., J.A. Loo, R.R. Ogorzalek, M. Busman and H.R. Udseth. 1991. Principle and practice of electrospray ionization – mass spectrometry for large peptide and proteins. **Mass.Spectrom.Rev**. 10:359 – 452

Sonenshine, D.E. 1991. Biology of ticks. **Oxford University Press**. New York. 141 – 158

Straub, R. and R.D. Voyksnere. 1993. Determination of penicillin G, ampicillin, amoxicillin, cloxacillin and cephapirin by high – performance liquid chromathography – electrospray mass spectrometry. **J.Chromatogr**. 647:167 – 181

Sugino, M., S. Imamura, A. Mulenga, K. Ohashi and M. Onuma. 2003. A serine proteinase inhibitor (serpin) from ixodid tick Haemaphysalis longicornis; cloning and preliminary assessment of it's suitability as a candidate for a tick vaccine. **Vaccine**. 21:2844 – 2851

Sutherst, R.W., R.H. Wharton and K.B.W. Utech. 1978. Guide to studies on tick ecology. **Technical paper No.14**. CSIRO.Aus.Div.Entomol

______, G.F. Maywald, J.D. Kerr and D.A. Stegeman. 1983. The effect of cattle tick (*Boophilus microplus*) on the growth of *Bos indicus* x *B. taurus* steers. **Aus.J.Agric.Res**. 34:317 – 327

Tanaka, K., H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida and T. Matsuo. 1988. Protein analyses up to m/z 100,000 by laser ionization TOF – MS. **Rapid Commun. Mass Spectrum**. 2:151 – 153

______, Y. Ido, S. Akita, Y. Yoshida and T. Yoshida. 1987. Detection of high mass molecular by laser desorption time – of – flight mass spectrometry. **In Proc.2nd.Japan – China Joint Symp. Mass Spectrum**. (eds. Matsuda, H, and Xiao – Tian, L.). 185 – 188

Tellam, R.L., D. Smith and D.H. Kemp. 1992. Vaccination against ticks. In: Yong, W.K. (Ed.), **Animal Parasite Control Using Biotechnology**. CRC Press, Boca Raton. 303 – 381

Till, W.M. 1961. A Contibution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus* Neumann. **Mem.Entomol.Soc.Afr**. 6:1 – 12

Towbin, H. and J. Gordon. 1984 Immunoblotting and dot immunobinding – Current status and outlook. **J. Immunol.Method**. 72:313 – 340

______, T. Staehelin and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrilamide gels to nitrocellulose sheets: Procedure and some applications. **Proc.Natl.Acad.Sci**. 76:4350 – 4354

Trager, W. 1939. Acquired immunity to tick. **J.Parasitol**. 25:57 – 81

Trimnell, A.R., R.S. Hails and P.A. Nuttall. 2002. Dual action ecotoparasite vaccine targeting ‘exposed’ and ‘concealed’ antigens. **Vaccine**. 20:3560 – 3568

Unlu, M., M.E. Morgan and J.S. Minder. 1997. Difference gel electrophoresis: a single gel method to detecting changes in protein extracts. **Electrophoresis**. 18:2071 – 2077

Untalan, P.M., F.D. Guerrero, L.R. Haines and T.W. Pearson. 2005. Proteome analysis of abundantly expressed protein form unfed larvae of the cattle tick, *Boophilus microplus*. **Insect Biochem.Mole Biol**.35:141 – 151

Wikel, S.K. 1996. Host immunity to ticks. **Annual review of Entomology**. 41:1 – 22

Willadsen, P. 1990. Perspective form subunit vaccines for the control of ticks. **Parasitologia**. 36:195 – 200

Willadsen, P. 1997. Novel vaccines for ectoparasites. **Vet.Parasit**. 71:209 – 222

______. 2004. Anti – tick vaccines. **Parasitology**. 129:S1 – S21

______, D. Smith, G.S. Cobon and R.V. Mckenna. 1996. Comparative vaccination of cattle against *Boophilus microplus* with recombinant antigen Bm86 alone or in combination with recombinant Bm91. **Parasitol.Immunol**. 18:241 – 246

______, G.A. Riding, R.V. McKenna, D.H. Kemp, R.L. Tellam, J.N. Nielsen, J. Lahnstein, G.S. Conbon and G.H. Gough. 1989. Immunologic control of a parasitic arthropod. Identification of a protective antigen from *Boophilus microplus* . **J.Immunol**. 143:1346 – 1351

______, G.M. Wood and G.A. Riding. 1979. The relation between skin histamine concentration, histamine sensitivity and the resistance of cattle to the tick, *Boophilus microplus*. **Zeishrift fur Parasitenkunde**. 59:87 – 93

______, P. Bird, G.S. Conbon and J. Hungerford. 1995. Commercialization of a recombinant vaccine against *Boophilus microplus*. **Parasitology**. 110:843 – 850

______, R.V. Mckenna and G.A. Riding. 1988. Isolation from the cattle tick *Boophilus microplus*, of antigenic material capable of eliciting a protective immunological response in the bovine host. **Int.J.Parasitol**. 18:183 – 189

Willoughby, R.C. E.W. Sheehan and S. Mitrovich. 1997. **A Global View of LC/MS, How to Solve Your Most Challenging Analytical Problem** .Global View Publishing: Pittsburgh. 500 pages

Wong, J.Y.M. and J.P. Opdebeeck. 1989. Protective efficacy of antigens solublized form gut membranes of the cattle tick, *Boophilus microplus*. **Immunol**. 66:149 – 155

Xu,Y., J.F. Bruno and B.J. Luft. 2005. Identification of a novel tick salivary gland proteins for vaccine development. **Biochem.Biophyisic.Res.Com**. 326:901 - 904

APPENDICES

Appendix A

The presentation of protein identification lists table form gel number 1 (G1) to gel number 11 (G11)

Appendix Table A1 Protein identification data form LCMS analysis. This table presented data of gel number 1 (G1) which found protein bands in tick's gut protein.

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	30	40519	6.4	K.EGGPPKSKPK.K
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	28	85695	7.94	K.AATPSPKQPK.E
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	27	112787	4.31	K.LETKGLFMSR.L
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	25	406489	5.99	K.EPGQLQQLLR.D
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	23	20283	7.79	R.GNGGGGGGGGGSR.G
gi|156366213	predicted protein [Nematostella vectensis]	22	56054	8.98	K.GAAGSRPPSPER.D
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	20	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	20	6635	9.81	K.TITDNKEMK.T
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	19	22842	5.43	M.ASLTIPKAPK.K
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	19	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|115618667	PREDICTED: similar to fibroblast growth factor receptor 4, partial [Strongylocentrotus purpuratus]	19	83654	9.5	R.GGNSQHAMPGK.G + Oxidation (M)
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	19	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	19	48763	5.94	K.LGGEKPAKPK.E
gi|170586404	F-box domain containing protein [Brugia malayi]	19	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	19	138255	6.59	R.GKLIQLQPK.T

Appendix Table A1 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	19	38467	9.67	K.IKSNPIQPK.F
gi|241116846	ribonucleoside-diphosphate reductase large chain, putative [Ixodes scapularis]	19	43524	8.07	K.EFIHSIMLFLDNVLEDFINK.A + Oxidation (M)
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	19	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|156321438	hypothetical protein NEMVEDRAFT_v1g225319 [Nematostella vectensis]	18	26748	7.85	K.LVMRHAGLLR.K + Oxidation (M)
gi|196012718	hypothetical protein TRIADDRAFT_60295 [Trichoplax adhaerens]	18	78546	6.06	R.LLDTTTTDAK.E
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	18	194679	8.93	R.RVSEGNAPHSK.N
gi|221106965	PREDICTED: similar to CG31809 CG31809-PB, partial [Hydra magnipapillata]	18	28677	7.74	K.LNVLLICKNTDK.L
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	18	126171	7.14	R.IITLLRQIIR.T
gi|156554877	PREDICTED: similar to LD15947p [Nasonia vitripennis]	17	105231	6.08	R.DLLPLVASSNK.A
gi|189237783	PREDICTED: similar to 2-phosphodiesterase [Tribolium castaneum]	17	65715	8.33	R.DKSTWVQVANTFR.Y
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	17	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|242020432	serine protease inhibitor, putative [Pediculus humanus corporis]	17	40191	6.34	R.DHDEGVQILR.S
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	17	52382	4.11	K.NGGGDDDDDDDEGNGFGRGGGGGR.G
gi|156542955	PREDICTED: similar to ENSANGP00000009960 [Nasonia vitripennis]	17	68768	8.57	R.HAMQQGKILR.D
gi|156399381	predicted protein [Nematostella vectensis]	16	410087	4.74	K.EWHTVQIIR.D

Appendix Table A1 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156408383	predicted protein [Nematostella vectensis]	16	56096	8.67	R.HSQVNSKPER.G
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	16	159499	5.87	K.STHDVAVKLLR.D
gi|195996053	hypothetical protein TRIADDRAFT_51865 [Trichoplax adhaerens]	16	147604	4.94	R.DVTETLSTDV.-
gi|170063504	hypothetical protein CpipJ_CPIJ016792 [Culex quinquefasciatus]	16	5669	11.59	R.VQRSSNSK.T
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	16	110364	5.32	K.MNPFNVPK.T
gi|270003804	hypothetical protein TcasGA2_TC003083 [Tribolium castaneum]	16	14595	11.28	K.EDAGVYYVMIK.S
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	16	67527	9.56	K.AQINVALKIIR.N
gi|156400082	predicted protein [Nematostella vectensis]	16	62244	9.78	K.EDKLLDHR.D
gi|170059237	conserved hypothetical protein [Culex quinquefasciatus]	16	117287	5.27	K.TLKTIGGYVK.N
gi|170072833	wd-repeat protein [Culex quinquefasciatus]	16	219090	9.03	R.NNSSRPLRR.S
gi|170037064	phenylalanyl-tRNA synthetase beta chain [Culex quinquefasciatus]	15	38924	9.08	R.IPKTLPATIHIAR.Q
gi|110749290	PREDICTED: similar to CG2004-PA [Apis mellifera]	15	41671	8.52	K.YHSTSVITQGDSWIPNYMINK.T
gi|91081233	PREDICTED: similar to hormone-sensitive lipase [Tribolium castaneum]	15	84776	5.09	K.YKSKPPAR.G
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	15	165556	6.1	K.MVLPRLQIIR.G
gi|170041667	serine/arginine rich splicing factor [Culex quinquefasciatus]	15	20743	11.57	R.NGGGGGRSER.G

Appendix Table A1 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|118781274	Toll-like receptor (AGAP010669-PA) [Anopheles gambiae str. PEST]	14	116352	6.2	K.DVQTTDNMR.M
gi|221118591	PREDICTED: similar to predicted protein [Hydra magnipapillata]	14	123001	8.94	R.WKNDSCLR.Q
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	14	34876	7.64	R.RGTDNAAVFK.F
gi|242002008	Niemann-Pick type C1 domain-containing protein, putative [Ixodes scapularis]	14	138159	6.7	R.QVPNLAPLPLDPR.K
gi|170029427	o-linked N-acetyl glucosamine transferase [Culex quinquefasciatus]	14	94887	9.3	K.DKLIEHIQLQEMATVTTAVVR.S

Appendix Table A2 Protein identification data form LCMS analysis. This table presented data of gel number 1 (G1) which found protein bands in Bm95 proteins.

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268558454	Hypothetical protein CBG18883 [Caenorhabditis briggsae]	33	302891	4.66	R.CEIFNDVEKVETACIVER.I
gi|156546770	PREDICTED: similar to rap55 [Nasonia vitripennis]	22	61343	9.32	R.NQQPGGAGVKPK.N
gi|242013855	conserved hypothetical protein [Pediculus humanus corporis]	20	376680	5.3	K.CENMLAMKDETELNLNNR.I + Oxidation (M)
gi|256070183	hypothetical protein [Schistosoma mansoni]	18	45798	7.19	K.KPLTRNKPVK.T
gi|119370319	T-box protein TbxE [Mnemiopsis leidyi]	17	27268	8.27	K.NSSAHPSCLK.E
gi|256071803	protein kinase [Schistosoma mansoni]	17	81688	8.89	K.ISPPRLPVDQDCHEMWSK.R + Oxidation (M)
gi|256079403	transient receptor potential cation channel subfamily m member [Schistosoma mansoni]	17	195145	8.89	R.SRVPTGSFSSR.Q
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	16	47623	7.81	R.LLVASQPEVLI.-
gi|156379770	predicted protein [Nematostella vectensis]	16	39226	8.54	K.DEVKHIQGGAK.A
gi|241561644	synembryn, putative [Ixodes scapularis]	16	14334	4.73	K.LARGGVVQPAR.V
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	15	194679	8.93	R.RVSEGNAPHSK.N
gi|156362206	predicted protein [Nematostella vectensis]	15	34377	5.63	R.TPGSEGHRIVK.T
gi|256070570	ubiquinone biosynthesis protein [Schistosoma mansoni]	15	20679	6.07	-.MLNHPDGCR.I
gi|170577046	trafficking protein particle complex 5 [Brugia malayi]	15	21711	9.08	K.LLNMLMFVK.G + Oxidation (M)
gi|221090805	PREDICTED: similar to putative DNA topoisomerase I [Hydra magnipapillata]	15	50216	8.9	K.MDPKNHNIGR.T

Appendix Table A2 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|193668032	PREDICTED: similar to AGAP007175-PA [Acyrthosiphon pisum]	15	62018	8.64	K.EDFDSEDDK.N
gi|158285848	AGAP007335-PA [Anopheles gambiae str. PEST]	14	121740	6.04	K.VQLMASEK.K
gi|156384835	predicted protein [Nematostella vectensis]	14	18421	9.65	K.SGAKQSPFFR.K
gi|156393510	predicted protein [Nematostella vectensis]	14	14503	10.08	R.NKTQDIIHR.T
gi|115739527	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	14	113994	6.2	R.MPPHLMMDK.G
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	14	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|50053648	cytochrome P450, family 51, subfamily A, polypeptide 1 [Strongylocentrotus purpuratus]	14	56608	7.11	K.FSYVPFGAGR.H
gi|156541190	PREDICTED: similar to signal recognition particle receptor alpha subunit [Nasonia vitripennis]	14	69406	8.39	K.KQNKPIEVPK.V
gi|170046504	nuclear pore complex protein nup214 [Culex quinquefasciatus]	14	179009	9.14	K.ADGVKAESK.G
gi|158297068	AGAP008093-PA [Anopheles gambiae str. PEST]	14	59258	6.13	R.LLLDRLGPAR.D
gi|3660698	period [Cydia pomonella]	14	39618	5.77	R.ILNEVPTRPAELVKQQMTK.R + Oxidation (M)
gi|34597030	RNA polymerase II largest subunit [Ballophilus australiae]	13	10360	4.97	R.FQASQVQP.-
gi|221123092	PREDICTED: similar to predicted protein [Hydra magnipapillata]	13	79325	8.78	R.VEMFNAGMQG.- + Oxidation (M)
gi|156552303	PREDICTED: similar to KRP170 [Nasonia vitripennis]	13	126113	6.61	R.VDEQKHLQK.L
gi|157713480	nicotinic acetylcholine receptor beta subunit [Haemonchus contortus]	13	55417	6.19	R.TLGAQEYSVR.V

Appendix Table A2 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	13	138300	5.84	K.LMVKYDDIK.I
gi|194068379	actin-related protein 2/3 [Saccostrea kegaki]	12	41027	8.25	K.EAGTMSAMNR.F + 2 Oxidation (M)
gi|170523096	cytochrome b [Cixius similis]	12	17677	8.26	K.DIMGFMFIMFMLTILVLK.N + 3 Oxidation (M)
gi|242005496	conserved hypothetical protein [Pediculus humanus corporis]	12	117349	5.6	K.LLPKSIEPVK.M
gi|242007326	conserved hypothetical protein [Pediculus humanus corporis]	11	130065	8.43	K.DLVHEMVLK.K + Oxidation (M)
gi|62122567	beta-catenin [Dugesia japonica]	11	79078	5.69	R.NLSMISPK.C + Oxidation (M)
gi|242007732	Nuclear transcription factor Y subunit alpha, putative [Pediculus humanus corporis]	11	44987	8	K.TPVQVKPTVR.L
gi|189241371	PREDICTED: similar to MGC52894 protein [Tribolium castaneum]	11	70503	7.22	R.ERAPLVLVFVNNDPFLIVR.S
gi|221128657	PREDICTED: similar to predicted protein [Hydra magnipapillata]	11	55989	5.93	R.RAFPSAEK.F
gi|37811697	rieske iron-sulfur protein [Tigriopus californicus]	10	25367	8.76	R.SVGVQWTK.T
gi|268562146	Hypothetical protein CBG20501 [Caenorhabditis briggsae]	10	171712	8.88	R.VYFPSMALR.K + Oxidation (M)
gi|193610709	PREDICTED: similar to mKIAA2037 protein [Acyrthosiphon pisum]	10	78775	8.87	K.IMDLASEK.S
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	13	138300	5.84	K.LMVKYDDIK.I
gi|194068379	actin-related protein 2/3 [Saccostrea kegaki]	12	41027	8.25	K.EAGTMSAMNR.F + 2 Oxidation (M)
gi|170523096	cytochrome b [Cixius similis]	12	17677	8.26	K.DIMGFMFIMFMLTILVLK.N + 3 Oxidation (M)

Appendix Table A2 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|221117402	PREDICTED: similar to CG15040 CG15040-PA [Hydra magnipapillata]	10	335193	9.28	R.ENINASEK.D
gi|14140052	membrane aminopeptidase H11-4, isoform 4 [Haemonchus contortus]	10	111340	6.55	R.DNHKVFSPR.T
gi|290561074	3,2-trans-enoyl-CoA isomerase, mitochondrial [Lepeophtheirus salmonis]	10	30933	9.44	R.DKVQSSIK.S
gi|157108793	hypothetical protein AaeL_AAEL005105 [Aedes aegypti]	10	86374	7.28	K.SRQDSSPK.D
gi|48994490	octopine dehydrogenase [Todaropsis eblanae]	7	29648	5.61	K.DGTMEIITSRPNMITNDASK.A + Oxidation (M)

Appendix Table A3 Protein identification data form LCMS analysis. This table presented data of gel number 1 (G1) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|193622606	PREDICTED: similar to Cyclin B3 CG5814-PA [Acyrthosiphon pisum]	31	46245	8.29	K.MCNKVIETK.E + Oxidation (M)
gi|157120954	hypothetical protein AaeL_AAEL009188 [Aedes aegypti]	28	26704	7.77	K.ITVLEPKPK.V
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	26	225555	5.77	K.DQVGQVNPPK.Y
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	25	406489	5.99	K.EPGQLQQLLR.D / R.SIPNEVLEDSK.I
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	24	22059	6.92	M.ENKLDSYR.K
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	24	67527	9.56	K.AQINVALKIIR.N
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	22	112787	4.31	K.LETKGLFMSR.L
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	21	194679	8.93	R.RVSEGNAPHSK.N
gi|60691514	SJCHGC05193 protein [Schistosoma japonicum]	21	9688	9.7	R.DKQPNGPLR.S
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	21	48812	8.57	K.SQKYGHVK.S
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	21	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F

Appendix Table A3 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|115659	RecName: Full=Short-chain collagen C4	21	36445	4.58	K.GDTGAPGPQGPK.G
gi|156547814	PREDICTED: similar to GA19427-PA [Nasonia vitripennis]	21	73676	5.7	R.RGSGQEEMTK.D + Oxidation (M)
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	20	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	20	165126	9.32	R.ILLDQRVGAPR.Y
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	20	165556	6.1	K.MVLPRLQIIR.G
gi|170574856	Protein kinase domain containing protein [Brugia malayi]	20	26666	9.93	R.DLDHNDQLLR.I
gi|156366213	predicted protein [Nematostella vectensis]	19	56054	8.98	K.GAAGSRPPSPER.D
gi|256084556	ADP-ribosylation factor interacting protein [Schistosoma mansoni]	19	38945	8.31	K.IKLPNAENGVSH.- / K.QMIEEKLGTASVTCDQELENR.I + Oxidation (M)
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	19	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|158295094	AGAP005971-PA [Anopheles gambiae str. PEST]	19	78047	5.5	R.SRMDYEIPK.Q
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	19	20283	7.79	R.GNGGGGGGGGSR.G
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	19	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	19	6635	9.81	K.TITDNKEMK.T
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	19	9092	9.51	-.MECLRSVLTIAGK.A
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	19	16163	9.54	-.MAALLLQLNSCSR.K

Appendix Table A3 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	13	138300	5.84	K.LMVKYDDIK.I
gi|194068379	actin-related protein 2/3 [Saccostrea kegaki]	12	41027	8.25	K.EAGTMSAMNR.F + 2 Oxidation (M)
gi|170523096	cytochrome b [Cixius similis]	12	17677	8.26	K.DIMGFMFIMFMLTILVLK.N + 3 Oxidation (M)
gi|242005496	conserved hypothetical protein [Pediculus humanus corporis]	12	117349	5.6	K.LLPKSIEPVK.M
gi|242007326	conserved hypothetical protein [Pediculus humanus corporis]	11	130065	8.43	K.DLVHEMVLK.K + Oxidation (M)
gi|62122567	beta-catenin [Dugesia japonica]	11	79078	5.69	R.NLSMISPK.C + Oxidation (M)
gi|242007732	Nuclear transcription factor Y subunit alpha, putative [Pediculus humanus corporis]	11	44987	8	K.TPVQVKPTVR.L
gi|189241371	PREDICTED: similar to MGC52894 protein [Tribolium castaneum]	11	70503	7.22	R.ERAPLVLVFVNNDPFLIVR.S
gi|221128657	PREDICTED: similar to predicted protein [Hydra magnipapillata]	11	55989	5.93	R.RAFPSAEK.F
gi|37811697	rieske iron-sulfur protein [Tigriopus californicus]	10	25367	8.76	R.SVGVQWTK.T
gi|268562146	Hypothetical protein CBG20501 [Caenorhabditis briggsae]	10	171712	8.88	R.VYFPSMALR.K + Oxidation (M)
gi|193610709	PREDICTED: similar to mKIAA2037 protein [Acyrthosiphon pisum]	10	78775	8.87	K.IMDLASEK.S
gi|221117402	PREDICTED: similar to CG15040 CG15040-PA [Hydra magnipapillata]	10	335193	9.28	R.ENINASEK.D
gi|14140052	membrane aminopeptidase H11-4, isoform 4 [Haemonchus contortus]	10	111340	6.55	R.DNHKVFSPR.T
gi|290561074	3,2-trans-enoyl-CoA isomerase, mitochondrial [Lepeophtheirus salmonis]	10	30933	9.44	R.DKVQSSIK.S
gi|157108793	hypothetical protein AaeL_AAEL005105 [Aedes aegypti]	10	86374	7.28	K.SRQDSSPK.D
gi|48994490	octopine dehydrogenase [Todaropsis eblanae]	7	29648	5.61	K.DGTMEIITSRPNMITNDASK.A + Oxidation (M)

Appendix Table A4 Protein identification data form LCMS analysis. This table presented data of gel number 2 (G2) which found protein bands in tick's gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	41	42046	8.57	-.MAALTKGVFIVAAK.R
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	28	66947	8.64	K.ILKNLAGKPK.I
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	28	34737	8.06	K.IINLAQGKPK.G
gi|242023447	conserved hypothetical protein [Pediculus humanus corporis]	28	48829	8.68	K.DFIENSLR.K
gi|268572719	Hypothetical protein CBG13254 [Caenorhabditis briggsae]	28	55921	6.59	K.NLMEITQK.F + Oxidation (M)
gi|158294544	AGAP005651-PA [Anopheles gambiae str. PEST]	28	47687	5.78	R.NIFEDLNK.-
gi|242021826	Angiotensin-converting enzyme precursor, putative [Pediculus humanus corporis]	27	81610	9.34	K.NYILLSNR.A
gi|225711702	Transmembrane and coiled-coil domain-containing protein 1 [Caligus rogercresseyi]	27	21647	9.75	R.DLMLVKMK.S + Oxidation (M)
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	27	23318	10.58	K.ENQKPIKPK.T
gi|193652752	PREDICTED: similar to exosome complex exonuclease RRP44 [Acyrthosiphon pisum]	26	94487	6.53	M.DFLENLSR.Q
gi|268578537	Hypothetical protein CBG13997 [Caenorhabditis briggsae]	25	25220	9.59	K.LLKIEQLLL.-
gi|289743875	hypothetical secreted peptide precursor [Glossina morsitans morsitans]	25	7121	9.89	R.NFLNILMK.F
gi|242011525	conserved hypothetical protein [Pediculus humanus corporis]	25	101669	9.74	K.DMIIETKK.K + Oxidation (M)
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	25	112787	4.31	K.LETKGLFMSR.L
gi|158287207	AGAP011349-PA [Anopheles gambiae str. PEST]	24	65162	9.04	R.SERLPQQPK.V
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	41	42046	8.57	-.MAALTKGVFIVAAK.R
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	28	66947	8.64	K.ILKNLAGKPK.I

Appendix Table A4 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|241618034	hypothetical protein IscW_ISCW010219 [Ixodes scapularis]	23	30351	5.98	R.ACGNLFKLDAFQK.L
gi|242019756	Caldesmon, putative [Pediculus humanus corporis]	23	104501	9.12	R.MSRELTQK.V
gi|189233623	PREDICTED: similar to fibroblast growth factor receptor [Tribolium castaneum]	23	109725	6.63	K.MIDIETVR.A + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	23	48763	5.94	K.LGGEKPAKPK.E
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	23	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|157125808	membrane traffic protein [Aedes aegypti]	22	51628	8.84	R.MAALVCIPQTTSK.R
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	21	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	21	22059	6.92	M.ENKLDSYR.K
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	21	194679	8.93	R.RVSEGNAPHSK.N
gi|156363128	predicted protein [Nematostella vectensis]	21	37562	6.31	K.FPVRPLIIK.T
gi|156383423	predicted protein [Nematostella vectensis]	20	11177	5.05	R.SIDDFWTGVP.-
gi|3608420	cyclin A [Dreissena polymorpha]	19	47955	5.76	R.VQPSRAAKPK.V
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	19	225555	5.77	K.DQVGQVNPPK.Y
gi|72058953	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	18	69619	6.02	R.LQALINKPK.V
gi|196004448	hypothetical protein TRIADDRAFT_23982 [Trichoplax adhaerens]	18	65010	8.81	K.LTLCMLGGWAISYLSILKGIK.S
gi|241618034	hypothetical protein IscW_ISCW010219 [Ixodes scapularis]	23	30351	5.98	R.ACGNLFKLDAFQK.L
gi|242019756	Caldesmon, putative [Pediculus humanus corporis]	23	104501	9.12	R.MSRELTQK.V

Appendix Table A4 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268536920	Hypothetical protein CBG05472 [Caenorhabditis briggsae]	18	37769	8.85	K.GGTGFGVMDLLNQTVSQSIFGPR.R
gi|115659	RecName: Full=Short-chain collagen C4	18	36445	4.58	K.GDTGAPGPQGPK.G
gi|268579035	C. briggsae CBR-CKA-2 protein [Caenorhabditis briggsae]	18	49741	5.75	R.NEPNKVLLR.V
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	18	16163	9.54	-.MAALLLQLNSCSR.K
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	18	22842	5.43	M.ASLTIPKAPK.K
gi|56756192	SJCHGC09241 protein [Schistosoma japonicum]	18	49876	5.38	K.VENGPRKPK.E
gi|60691514	SJCHGC05193 protein [Schistosoma japonicum]	18	9688	9.7	R.DKQPNGPLR.S
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	17	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	17	38467	9.67	K.IKSNPIQPK.F
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	16	115140	8.72	K.MGPPMQGR.G + Oxidation (M)
gi|268552739	Hypothetical protein CBG17702 [Caenorhabditis briggsae]	16	55277	9.63	R.DSAITGEQINEDK.I
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	16	9092	9.51	-.MECLRSVLTIAGK.A
gi|156401368	predicted protein [Nematostella vectensis]	16	55046	6.23	K.DISQTLANFK.K
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	16	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|225709606	Abhydrolase domain-containing protein 11 [Caligus rogercresseyi]	15	36210	8.26	K.CDYLREPDIEEVSSLFPNAK.F
gi|268536920	Hypothetical protein CBG05472 [Caenorhabditis briggsae]	18	37769	8.85	K.GGTGFGVMDLLNQTVSQSIFGPR.R
gi|115659	RecName: Full=Short-chain collagen C4	18	36445	4.58	K.GDTGAPGPQGPK.G

Appendix Table A4 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170586408	dTDP-4-dehydrorhamnose 3,5-epimerase [Brugia malayi]	15	21796	5.82	K.LVSVISGEIYDVAVDIRSMSK.T
gi|268533246	Hypothetical protein CBG20958 [Caenorhabditis briggsae]	15	42124	9.4	K.IGEHVVIWTDNPDKMLEIR.T + Oxidation (M)
gi|241304593	hypothetical protein IscW_ISCW017719 [Ixodes scapularis]	14	35769	8.36	R.NQTGQAVCFR.C
gi|241561644	synembryn, putative [Ixodes scapularis]	14	14334	4.73	K.LARGGVVQPAR.V
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	14	406489	5.99	K.EPGQLQQLLR.D

Appendix Table A5 Protein identification data form LCMS analysis. This table presented data of gel number 2 (G2) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|34500282	Bm95 protein [Rhipicephalus microplus]	56	67154	6.44	R.NAECEVVPGAEDDFVCK.C
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	39	406489	5.99	K.EPGQLQQLLR.D
gi|268552739	Hypothetical protein CBG17702 [Caenorhabditis briggsae]	31	55277	9.63	R.DSAITGEQINEDK.I
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	27	42046	8.57	-.MAALTKGVFIVAAK.R
gi|156392182	predicted protein [Nematostella vectensis]	27	57541	9.23	R.GSAPPPPPAR.M
gi|91081233	PREDICTED: similar to hormone-sensitive lipase [Tribolium castaneum]	26	84776	5.09	K.YKSKPPAR.G
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	26	20283	7.79	R.GNGGGGGGGGGSR.G
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	25	99637	4.8	K.ALLNGLKAEPR.V
gi|15298096	green fluorescent protein [Montastraea cavernosa]	25	26002	6.09	K.LRMQGVVNGHK.F
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	24	48812	8.57	K.SQKYGHVK.S
gi|115659	RecName: Full=Short-chain collagen C4	24	36445	4.58	K.GDTGAPGPQGPK.G
gi|170047092	serrate protein [Culex quinquefasciatus]	24	419035	5.51	R.IRLGCTAR.Y
gi|261887729	RecQ helicase [Heliconius melpomene melpomene]	24	35690	8.28	K.GVEPNFAR.N / K.GREPNFAR.N
gi|170580994	U1 zinc finger family protein [Brugia malayi]	24	51370	7.07	K.IDGPIEFR.K
gi|110756444	PREDICTED: similar to CG33515-PA isoform 1 [Apis mellifera]	22	73958	6.23	K.TASNPVTLQIMYTPVCK.E + Oxidation (M)

Appendix Table A5 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	21	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|170586404	F-box domain containing protein [Brugia malayi]	21	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	21	150695	6.9	K.SCEYIKPK.E
gi|170523056	cytochrome b [Tachycixius pilosus]	21	16608	6.36	R.SIPNKLGGVIAL.-
gi|156546758	PREDICTED: similar to ATP-binding cassette sub-family A member 3, putative [Nasonia vitripennis]	20	187844	8.66	R.MPNEFYDVACK.S
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	20	6635	9.81	K.TITDNKEMK.T
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	20	34876	7.64	R.RGTDNAAVFK.F
gi|156408383	predicted protein [Nematostella vectensis]	19	56096	8.67	R.HSQVNSKPER.G
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	19	1154140	8.72	K.MGPPMQGR.G + Oxidation (M)
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	19	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|76154609	SJCHGC03612 protein [Schistosoma japonicum]	19	19151	6.23	K.SGAKFLHLAR.D

Appendix Table A5 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	19	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	19	78492	8.75	R.SPELLLQPK.T
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	19	174650	5.27	R.GAPGLSGLKGPK.G
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	19	112787	4.31	K.LETKGLFMSR.L
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	19	138300	5.84	K.LMVKYDDIK.I
gi|242025626	hypothetical protein Phum_PHUM617340 [Pediculus humanus corporis]	18	24213	5.13	R.IDGIEARFEPVK.H
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	18	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|115710072	PREDICTED: similar to Os01g0959000, partial [Strongylocentrotus purpuratus]	18	67831	9.49	R.FRTQPITVDEVK.K
gi|170591068	hypothetical protein Bm1_44155 [Brugia malayi]	18	23472	9.46	K.VFGWVTLPWTVK.Q
gi|110755833	PREDICTED: similar to Eukaryotic translation initiation factor 4B (eIF-4B) [Apis mellifera]	18	57467	8.53	K.EPVVLPTAPR.A
gi|76154409	SJCHGC07860 protein [Schistosoma japonicum]	18	14358	5.32	R.GPQYDGYGPPMG.-
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|170574609	Zinc finger, C2H2 type family protein [Brugia malayi]	18	46296	9.44	K.VKGDGLQGLSNER.K
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	18	34737	8.06	K.IINLAQGKPK.G
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	18	66947	8.64	K.ILKNLAGKPK.I

Appendix Table A5 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|158294505	AGAP005629-PA [Anopheles gambiae str. PEST]	17	75224	9.86	K.QNYTPPAR.I
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	17	225555	5.77	K.DQVGQVNPPK.Y
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	17	44167	8.9	K.MPGPTVVPIR.E + Oxidation (M)
gi|241708208	LIM domain-binding protein, putative [Ixodes scapularis]	17	60818	9.3	K.IPICSNCGSPIR.G
gi|221127100	PREDICTED: similar to conserved hypothetical protein, partial [Hydra magnipapillata]	17	86761	6.2	K.RVSPSSSAR.F

Appendix Table A6 Protein identification data form LCMS analysis. This table presented data of gel number 2 (G2) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1468981	angiotensin-converting enzyme-like protein [Rhipicephalus microplus]	319	75495	5.84	K.IAFLPFGYLLDK.W / K.DKDLPLEPDLTR.N / K.LSNEAASLDGYDNIK.S / K.SAWLSDYETENMTEIVDK.L + Oxidation (M)
gi|268558454	Hypothetical protein CBG18883 [Caenorhabditis briggsae]	44	302891	4.66	R.CEIFNDVEKVETACIVER.I
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	29	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	29	112787	4.31	K.LETKGLFMSR.L
gi|270009184	hypothetical protein TcasGA2_TC015840 [Tribolium castaneum]	28	95268	8.33	K.QDDIEIMTFRK.N + Oxidation (M)
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	27	24881	9.76	K.RADPGIFAWEIR.D
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	27	40519	6.4	K.EGGPPKSKPK.K
gi|268557876	Hypothetical protein CBG09395 [Caenorhabditis briggsae]	26	49977	9.47	R.LFKWNIWMIGILFK.I + Oxidation (M)
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	25	406489	5.99	K.EPGQLQQLLR.D
gi|157107906	lysosomal acid lipase, putative [Aedes aegypti]	25	46876	9.02	K.DALLTVPQLIR.K
gi|256073012	DNA-directed RNA polymerase I [Schistosoma mansoni]	25	201143	6.62	K.GGMVNAQQMSVALGQIELEGR.R + Oxidation (M)
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	24	22059	6.92	M.ENKLDSYR.K
gi|256087746	hypothetical protein [Schistosoma mansoni]	24	136058	8.48	R.TNWSPSLDLVGHRK.H
gi|13359096	Esterase-like protein (ESR-LP) [Bombyx mori]	23	34010	5.69	K.IWEELYPITN.-

Appendix Table A6 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|118783458	AGAP004126-PA [Anopheles gambiae str. PEST]	23	24643	9.64	K.SSISLGDSFELMK.T + Oxidation (M)
gi|242013855	conserved hypothetical protein [Pediculus humanus corporis]	23	376680	5.3	K.CENMLAMKDETELNLNNR.I + Oxidation (M)
gi|193700019	PREDICTED: hypothetical protein [Acyrthosiphon pisum]	23	83753	4.73	K.SEVLSSNNVAQSSNAEIILTK.T
gi|196009478	hypothetical protein TRIADDRAFT_58620 [Trichoplax adhaerens]	22	139335	6.39	R.INYGESEFLYQAADK.S
gi|268567117	Hypothetical protein CBG17912 [Caenorhabditis briggsae]	22	37983	9.16	R.QDIPMDHSTSHK.F + Oxidation (M)
gi|170580994	U1 zinc finger family protein [Brugia malayi]	21	51370	7.07	K.IDGPIEFR.K
gi|193620191	PREDICTED: similar to kinesin heavy chain subunit [Acyrthosiphon pisum]	21	104914	6.21	K.GIQYIPPSGKVYLFDK.V
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	21	48812	8.57	K.SQKYGHVK.S
gi|264667459	ribosomal protein L28 [Chrysomela tremulae]	21	16767	10.94	R.MIKYVVCI.-
gi|242011894	predicted protein [Pediculus humanus corporis]	21	210112	7.99	R.NEQAGAAAHIISENIK.M
gi|110764591	PREDICTED: similar to CG9886-like [Apis mellifera]	20	52963	8.56	K.ELVEETVPWIMAADK.Q + Oxidation (M)
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	20	126171	7.14	R.IITLLRQIIR.T
gi|170047092	serrate protein [Culex quinquefasciatus]	20	419035	5.51	R.IRLGCTAR.Y
gi|193603470	PREDICTED: similar to molting fluid carboxypeptidase A [Acyrthosiphon pisum]	20	58753	6.37	K.TADLVLPPSVAR.D

Appendix Table A6 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	20	99637	4.8	K.ALLNGLKAEPR.V
gi|270046366	arrestin [Triops granarius]	20	43758	8.99	K.LSLGALAGELVADVPLK.L
gi|170587102	GRIP domain containing protein [Brugia malayi]	20	52617	5.19	K.GLEEEMERLR.R + Oxidation (M)
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	20	52382	4.11	K.NGGGDDDDDDDEGNGFGRGGGGGR.G
gi|56752853	SJCHGC02799 protein [Schistosoma japonicum]	19	45027	6.05	R.QLDNSISFLLK.L
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	19	20283	7.79	R.GNGGGGGGGGGSR.G
gi|156321438	hypothetical protein NEMVEDRAFT_v1g225319 [Nematostella vectensis]	19	26748	7.85	K.LVMRHAGLLR.K + Oxidation (M)
gi|165928916	integrin beta 2 [Acropora millepora]	19	88216	5.29	K.KNDLLPGEIINGK.Y
gi|91080015	PREDICTED: similar to zormin CG33484-PD [Tribolium castaneum]	19	426398	6.26	K.LEAARIQALEAQKPK.I
gi|156543280	PREDICTED: hypothetical protein [Nasonia vitripennis]	19	129511	8.27	K.HSDSLLKILR.N
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	19	22842	5.43	M.ASLTIPKAPK.K
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	19	138255	6.59	R.GKLIQLQPK.T
gi|26984055	HSP70 [Chironomus tentans]	18	71412	6.05	K.MRETAEAYIGQK.V
gi|51849654	heat shock protein 70 [Chironomus yoshimatsui]	18	71674	5.76	K.MRETAEAYLGQK.V
gi|189239643	PREDICTED: similar to putative alcohol dehydrogenase [Tribolium castaneum]	18	26931	7.63	R.IRSFMSAFLDPK.S
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	20	99637	4.8	K.ALLNGLKAEPR.V

Appendix Table A6 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|89145849	inositol trisphosphate receptor [Aplysia californica]	18	318416	5.61	K.DQDVRMNELMK.L + 2 Oxidation (M)
gi|3426184	SEVENLESS [Anopheles sp.]	18	99503	8.47	R.CQNTSEDTQIIAPFPATCSK.V
gi|218117241	60S ribosomal protein L18a [Brachionus plicatilis]	18	21221	10.88	R.GHSIQIMRVEEIPANK.C
gi|270013398	hypothetical protein TcasGA2_TC011994 [Tribolium castaneum]	18	16832	9.71	R.FLDPSSLLNVVRTDDLWR.E
gi|193606029	PREDICTED: similar to Rab5 protein [Acyrthosiphon pisum]	18	23514	8.7	K.ELQRQATPGIVIALAGNK.L

Appendix Table A7 Protein identification data form LCMS analysis. This table presented data of gel number 3 (G3) which found protein bands in tick’s gut proteins.

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	40	22059	6.92	M.ENKLDSYR.K
gi|3426184	SEVENLESS [Anopheles sp.]	27	99503	8.47	R.CQNTSEDTQIIAPFPATCSK.V
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	26	75672	9.35	K.TLTFEEIRK.K
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	26	20283	7.79	R.GNGGGGGGGGGSR.G
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	25	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	24	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	23	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	23	194679	8.93	R.RVSEGNAPHSK.N
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	23	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	23	42046	8.57	-.MAALTKGVFIVAAK.R
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	23	48763	5.94	K.LGGEKPAKPK.E
gi|256075113	brefeldin A-inhibited guanine nucleotide-exchange protein [Schistosoma mansoni]	21	230732	5.6	R.LSKSHIGHFLGENEPYNLR.V
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|60691514	SJCHGC05193 protein [Schistosoma japonicum]	21	9688	9.7	R.DKQPNGPLR.S
gi|170586404	F-box domain containing protein [Brugia malayi]	21	53036	9.66	R.QNALLIQPK.A

Appendix Table A7 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	21	16163	9.54	-.MAALLLQLNSCSR.K
gi|221103497	PREDICTED: similar to predicted protein [Hydra magnipapillata]	20	19301	11.36	R.RHLPSLNQIR.F
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	19	9092	9.51	-.MECLRSVLTIAGK.A
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	19	56983	9.9	-.TLTHRVR.L
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	19	65748	9.67	R.TITHSPVK.S
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	19	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|156340946	hypothetical protein NEMVEDRAFT_v1g147631 [Nematostella vectensis]	19	41345	4.83	K.LLVIVSRPAR.L
gi|110749258	PREDICTED: similar to CG12524-PA [Apis mellifera]	18	87284	9.17	R.MSKMTQPNLDYIAPEIQQK.K + 2 Oxidation (M)
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|170584334	importin beta family protein 5 [Brugia malayi]	18	112436	5.45	-.MAAGAEAVAECLKR.T
gi|119370319	T-box protein TbxE [Mnemiopsis leidyi]	17	27268	8.27	K.NSSAHPSCLK.E
gi|156380891	predicted protein [Nematostella vectensis]	17	31522	10.15	K.IHMPPGSK.V + Oxidation (M)

Appendix Table A7 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|157125153	hypothetical protein AaeL_AAEL010109 [Aedes aegypti]	17	20735	6.54	K.TISTVMNT.- + Oxidation (M)
gi|268552739	Hypothetical protein CBG17702 [Caenorhabditis briggsae]	17	55277	9.63	R.DSAITGEQINEDK.I
gi|242005381	synaptic vesicular amine transporter, putative [Pediculus humanus corporis]	16	51848	8.54	K.TIFDVPNMENGRMSILLSSK.A + Oxidation (M)
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	16	48812	8.57	K.SQKYGHVK.S
gi|268554292	C. briggsae CBR-MYO-5 protein [Caenorhabditis briggsae]	16	219524	5.96	R.AAATETFRLR.N R.KVEGDLQHLQSEVEEALSDAK.V
gi|3892088	prophenoloxidase [Anopheles gambiae]	16	78684	6.3	K.FTVTLNAGANTIVR.R
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	16	110364	5.32	K.MNPFNVPK.T
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	16	112787	4.31	K.LETKGLFMSR.L
gi|157108284	serine protease inhibitor (serpin-2), putative [Aedes aegypti]	16	40339	6.03	R.MEMVHGVK.L + Oxidation (M)
gi|157123110	DNA-binding protein smubp-2 [Aedes aegypti]	15	79961	8.88	-.MIDAYSDASK.I
gi|256073891	hypothetical protein [Schistosoma mansoni]	15	61705	8.85	K.GNLDTSVSQTSISTASRSPSSK.L
gi|110756661	PREDICTED: similar to ubiquitin specific protease 34 [Apis mellifera]	15	389904	5.88	K.LLVPKLSEVMLSMMNVEAVMSR.I + 2 Oxidation (M)
gi|242015041	Hsp70-binding protein, putative [Pediculus humanus corporis]	15	40740	4.73	R.GSEEGLKEFIITDGFSVLLR.A
gi|241999928	kinesin-associated protein, putative [Ixodes scapularis]	15	77022	6.01	R.TPSGETASMAR.L + Oxidation (M)
gi|196010065	expressed hypothetical protein [Trichoplax adhaerens]	15	24977	6.97	R.TLHTTGPR.I

Appendix Table A7 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	15	47623	7.81	R.LLVASQPEVLI.-
gi|170580994	U1 zinc finger family protein [Brugia malayi]	15	51370	7.07	K.IDGPIEFR.K
gi|193622556	PREDICTED: similar to conserved hypothetical protein [Acyrthosiphon pisum]	15	63673	6.74	K.YYLDPLELLGERSFNEPR.E
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	14	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|115960782	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	14	88670	6.48	K.DLHLDLMRK.K + Oxidation (M)

Appendix Table A8 Protein identification data form LCMS analysis. This table presented data of gel number 3 (G3) which found protein bands in Bm95 proteins.

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|34500282	Bm95 protein [Rhipicephalus microplus]	56	67154	6.44	R.NAECEVVPGAEDDFVCK.C
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	37	34737	8.06	K.IINLAQGKPK.G
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	34	225555	5.77	K.DQVGQVNPPK.Y
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	29	66947	8.64	K.ILKNLAGKPK.I
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	25	112787	4.31	K.LETKGLFMSR.L
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	24	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	24	23318	10.58	K.ENQKPIKPK.T
gi|268537058	C. briggsae CBR-DPY-26 protein [Caenorhabditis briggsae]	22	141467	4.66	K.ATDAWTNYDVVKMIVNR.D
gi|193603470	PREDICTED: similar to molting fluid carboxypeptidase A [Acyrthosiphon pisum]	21	58753	6.37	K.TADLVLPPSVAR.D
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	21	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|115942988	PREDICTED: hypothetical protein, partial [Strongylocentrotus purpuratus]	21	231846	5.4	R.TCQTELASRDQSLLR.L
gi|15298096	green fluorescent protein [Montastraea cavernosa]	20	26002	6.09	K.LRMQGVVNGHK.F
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20	20283	7.79	R.GNGGGGGGGGGSR.G
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	20	126171	7.14	R.IITLLRQIIR.T
gi|290462563	Double-stranded RNA-specific editase 1 [Lepeophtheirus salmonis]	20	33112	9.32	K.NLCAESAIQSLVFQKCK.D

Appendix Table A8 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|193683581	PREDICTED: similar to Ubiquitin domain-containing protein UBFD1 [Acyrthosiphon pisum]	19	32663	5.19	K.EITTESVVEEQTTESADK.D
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	19	194679	8.93	R.RVSEGNAPHSK.N
gi|156363128	predicted protein [Nematostella vectensis]	19	37562	6.31	K.FPVRPLIIK.T
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	19	165556	6.1	K.MVLPRLQIIR.G
gi|170591600	Immunoglobulin I-set domain containing protein [Brugia malayi]	19	682327	5.1	K.TPVFDIMTPKAASDIR.V + Oxidation (M)
gi|157120760	hypothetical protein AaeL_AAEL001631 [Aedes aegypti]	18	53033	4.89	K.QITTSYSRPK.V
gi|195996053	hypothetical protein TRIADDRAFT_51865 [Trichoplax adhaerens]	18	147604	4.94	K.STHDVAVKLLR.D
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	18	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	17	9092	9.51	-.MECLRSVLTIAGK.A
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	17	16163	9.54	-.MAALLLQLNSCSR.K
gi|242012572	predicted protein [Pediculus humanus corporis]	17	70917	7.28	R.NQNALHMESLTIWERILGR.L + Oxidation (M)
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	17	406489	5.99	K.EPGQLQQLLR.D
gi|221130433	PREDICTED: similar to thioredoxin domain containing 11 [Hydra magnipapillata]	16	61790	9.12	R.LINRVNKPK.I
gi|256087925	pseudouridylate synthase [Schistosoma mansoni]	16	151995	5.86	R.ETITKHEKPR.E
gi|254934075	carbamoylphosphate synthetase [Eudonia spenceri]	16	109871	5.89	K.TFNPKGDVTIMTVDCGLK.Y

Appendix Table A8 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156366213	predicted protein [Nematostella vectensis]	16	56054	8.98	K.GAAGSRPPSPER.D
gi|193666940	PREDICTED: similar to Zn72D CG5215-PB [Acyrthosiphon pisum]	16	107882	9.01	K.WFQARLAVIANGPLIIR.I
gi|66509122	PREDICTED: similar to CG11594-PA, isoform A isoform 1 [Apis mellifera]	16	60540	6.4	K.AMGGSATMVKPR.S + 2 Oxidation (M)
gi|33591156	thioredoxin peroxidase [Ixodes ricinus]	16	19419	6.93	K.LTHPAPDFTGTAVVGGQFKDIK.L
gi|76155338	SJCHGC05221 protein [Schistosoma japonicum]	16	37634	5.89	-.PFQDEYKR.F
gi|115896473	PREDICTED: similar to MGC83212 protein [Strongylocentrotus purpuratus]	16	49626	10.28	K.LMKQDEYR.S
gi|115634798	PREDICTED: similar to ENSANGP00000007239 [Strongylocentrotus purpuratus]	16	305747	5.9	R.EKHDSSPGILR.Q
gi|268577465	C. briggsae CBR-LEC-7 protein [Caenorhabditis briggsae]	16	19615	6.02	-.MYIIKNPTIPTALEIVEPLK.A + Oxidation (M)
gi|268564260	Hypothetical protein CBG14871 [Caenorhabditis briggsae]	16	74721	5.82	K.QLAATEDAEPPLLSELLK.V
gi|196011459	hypothetical protein TRIADDRAFT_59472 [Trichoplax adhaerens]	16	580051	6.71	K.QQSMTAMAELPNTTGRTPGYK.A + Oxidation (M)
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	16	58245	8.67	K.VRYYQTPVR.D
gi|158287207	AGAP011349-PA [Anopheles gambiae str. PEST]	15	65162	9.04	R.SERLPQQPK.V
gi|156553640	PREDICTED: similar to Paxneb protein [Nasonia vitripennis]	15	26587	6.23	K.TLTEGQFLIAQTATKR.N
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	15	115140	8.72	**K.MGPPMQGR.G + Oxidation (M)**
gi|241162965	hypothetical protein IscW_ISCW016627 [Ixodes scapularis]	15	9338	11.95	R.ERPFGAGR.E

Appendix Table A8 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170035960	conserved hypothetical protein [Culex quinquefasciatus]	15	96405	6.65	K.LGGSNGFMRPGLGDMMDSPSIK.R + 2 Oxidation (M)
gi|221115200	PREDICTED: similar to conserved hypothetical protein [Hydra magnipapillata]	15	89278	9.2	K.IPDSWTVYKIAGK.E
gi|66557857	PREDICTED: similar to fat2 CG7749-PA, isoform A isoform 2, partial [Apis mellifera]	15	130998	4.87	R.VMVDDVNDNAPSFALPSYTVK.I + Oxidation (M)
gi|34880406	elongation factor 1-alpha [Liparthrum rumicis]	15	30209	7.93	K.MDSTEPPYSEPPFEEIK.K
gi|170052375	conserved hypothetical protein [Culex quinquefasciatus]	15	48084	6.85	**M.LSLDNFSVETYGMYR.I + Oxidation (M)**
gi|170035960	conserved hypothetical protein [Culex quinquefasciatus]	15	96405	6.65	K.LGGSNGFMRPGLGDMMDSPSIK.R + 2 Oxidation (M)

Appendix Table A9 Protein identification data form LCMS analysis. This table presented data of gel number 3 (G3) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1468981	angiotensin-converting enzyme-like protein [Rhipicephalus microplus]	85	75495	5.84	K.LSNEAASLDGYDNIK.S
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	32	34737	8.06	K.IINLAQGKPK.G
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	32	66947	8.64	K.ILKNLAGKPK.I
gi|268558454	Hypothetical protein CBG18883 [Caenorhabditis briggsae]	32	302891	4.66	R.CEIFNDVEKVETACIVER.I
gi|225709128	Mediator of RNA polymerase II transcription subunit 22 [Caligus rogercresseyi]	31	16687	5.12	K.MFLDLR.D
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	31	85695	7.94	K.AATPSPKQPK.E
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	30	40519	6.4	K.EGGPPKSKPK.K
gi|209489295	hypothetical protein Cbre_JD10.003 [Caenorhabditis brenneri]	29	59512	9.33	K.MFINNR.R
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	29	112787	4.31	K.LETKGLFMSR.L
gi|115659	RecName: Full=Short-chain collagen C4	28	36445	4.58	K.GDTGAPGPQGPK.G
gi|256087746	hypothetical protein [Schistosoma mansoni]	28	136058	8.48	R.TNWSPSLDLVGHRK.H
gi|268557876	Hypothetical protein CBG09395 [Caenorhabditis briggsae]	26	49977	9.47	R.LFKWNIWMIGILFK.I + Oxidation (M)
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	26	225555	5.77	K.DQVGQVNPPK.Y
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	26	174650	5.27	R.GAPGLSGLKGPK.G
gi|110765009	PREDICTED: similar to F02C12.1, partial [Apis mellifera]	25	36723	6.55	K.IDLQLKNNLNNIPIQK.S

Appendix Table A9 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156363128	predicted protein [Nematostella vectensis]	25	37562	6.31	K.FPVRPLIIK.T
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	24	22059	6.92	M.ENKLDSYR.K
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	23	44167	8.9	K.MPGPTVVPIR.E + Oxidation (M)
gi|56752637	SJCHGC09586 protein [Schistosoma japonicum]	22	47861	6.91	K.LQRLGENEGNLPK.E
gi|193716189	PREDICTED: similar to pigeon CG10739-PA [Acyrthosiphon pisum]	21	78496	8.21	R.YLPITISKHNIPLEIK.V
gi|110757623	PREDICTED: similar to lethal (2) 01424 CG3845-PB, isoform B [Apis mellifera]	21	105696	8.5	R.GLMDQYFK.R
gi|60691514	SJCHGC05193 protein [Schistosoma japonicum]	21	9688	9.7	R.DKQPNGPLR.S
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|72043691	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	21	72488	4.84	R.GIIQYMQKQVGDSSQLLGSTK.A + Oxidation (M)
gi|242013855	conserved hypothetical protein [Pediculus humanus corporis]	21	376680	5.3	K.CENMLAMKDETELNLNNR.I + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	20	23318	10.58	K.ENQKPIKPK.T
gi|157109998	hypothetical protein AaeL_AAEL005445 [Aedes aegypti]	20	98687	8.59	K.AVIVVSDNGK.T

Appendix Table A9 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1620935	Trp protein [Calliphora vicina]	20	133316	7.56	R.VAYVKFMK.K + Oxidation (M)
gi|115610200	PREDICTED: similar to ankyrin 2,3/unc44, partial [Strongylocentrotus purpuratus]	20	220930	5.15	K.LGLSYSSMK.K + Oxidation (M)
gi|115668585	PREDICTED: similar to PDZ domain containing 6 [Strongylocentrotus purpuratus]	20	107223	8.37	R.GLLPQGAAMK.S + Oxidation (M)
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	20	194679	8.93	R.RVSEGNAPHSK.N
gi|158297905	AGAP004765-PA [Anopheles gambiae str. PEST]	19	82488	6.3	K.MLKGEYTLK.A
gi|3608420	cyclin A [Dreissena polymorpha]	19	47955	5.76	R.VQPSRAAKPK.V
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	19	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	18	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|115896410	PREDICTED: similar to potassium channel modulatory factor 1 [Strongylocentrotus purpuratus]	18	47117	5.59	K.LGSLNLKEK.E
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|158451189	putative CAD trifunctional protein [Hyles lineata]	17	109387	6.04	K.EAVDNLKPVLQNK.N
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	17	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|242015404	conserved hypothetical protein [Pediculus humanus corporis]	17	207580	8.29	R.VASKLINEK.K
gi|156383423	predicted protein [Nematostella vectensis]	17	11177	5.05	R.SIDDFWTGVP.-
gi|156366213	predicted protein [Nematostella vectensis]	17	56054	8.98	K.GAAGSRPPSPER.D

Appendix Table A9 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156352327	predicted protein [Nematostella vectensis]	16	33127	8.96	R.AMQESMEAEKLILALK.H + Oxidation (M)
gi|99878583	NADH dehydrogenase subunit 1 [Thaumamermis cosgrovei]	16	33860	9.44	-.MMIKGAK.N + Oxidation (M)
gi|110749606	PREDICTED: similar to nucleoporin 107 [Apis mellifera]	16	103071	5.34	R.MFIEVR.C
gi|157057071	pH gated chloride channel [Sarcoptes scabiei type hominis]	16	57705	6.86	-.MFLKQK.L
gi|157126403	hypothetical protein AaeL_AAEL010489 [Aedes aegypti]	16	48836	6.1	R.MFLVER.K

Appendix Table A10 Protein identification data form LCMS analysis. This table presented data of gel number 4 (G4) which found protein bands in tick’s gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268558454	Hypothetical protein CBG18883 [Caenorhabditis briggsae]	47	302891	4.66	R.CEIFNDVEKVETACIVER.I
gi|270013886	hypothetical protein TcasGA2_TC012552 [Tribolium castaneum]	45	92073	9.01	R.MSSSGVSEMQFSMRSR.T + Oxidation (M)
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	43	42046	8.57	-.MAALTKGVFIVAAK.R
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	33	66947	8.64	K.ILKNLAGKPK.I
gi|170054451	general odorant-binding protein 56d [Culex quinquefasciatus]	28	14381	8.7	K.TVVEKLSIDHDK.S
gi|1515081	NADH dehydrogenase subunit 5 (ND5) [Carabus yaconinus]	27	41342	8.53	K.SSSLMMQFMQNNNMK.I + 2 Oxidation (M)
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	27	23318	10.58	K.ENQKPIKPK.T
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	25	225555	5.77	K.DQVGQVNPPK.Y
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	24	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	24	34737	8.06	K.IINLAQGKPK.G
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	23	52382	4.11	K.NGGGDDDDDDEGNGFGRGGGGGR.G
gi|114155295	NADH dehydrogenase subunit 5 [Carabus yaconinus yaconinus]	23	41326	8.52	K.SSSLVMQFYQNNNMK.I + 2 Oxidation (M)
gi|242013855	conserved hypothetical protein [Pediculus humanus corporis]	22	376680	5.3	K.CENMLAMKDETELNLNNR.I + Oxidation (M)
gi|156379770	predicted protein [Nematostella vectensis]	22	39226	8.54	K.DEVKHIQGGAK.A
gi|221130433	PREDICTED: similar to thioredoxin domain containing 11 [Hydra magnipapillata]	22	61790	9.12	R.LINRVNKPK.I
gi|268558454	Hypothetical protein CBG18883 [Caenorhabditis briggsae]	47	302891	4.66	R.CEIFNDVEKVETACIVER.I

Appendix Table A10 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156363128	predicted protein [Nematostella vectensis]	21	37562	6.31	K.FPVRPLIIK.T
gi|158297905	AGAP004765-PA [Anopheles gambiae str. PEST]	21	82488	6.3	K.MLKGEYTLK.A
gi|196006884	hypothetical protein TRIADDRAFT_7238 [Trichoplax adhaerens]	20	15711	9.38	K.TAAKGSPFFR.K
gi|156554020	PREDICTED: similar to lipase [Nasonia vitripennis]	19	39901	8.45	K.IIIHGFGGGRNLAPSTDLR.K
gi|254933957	carbamoylphosphate synthetase [Euclemensia bassettella]	19	109474	6.04	K.WLESYTHSSITFEILKGAK.Q
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	19	56983	9.9	-.TLTHRVR.L
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	19	65748	9.67	R.TITHSPVK.S
gi|289177039	cytochrome P450 4AB8 [Nasonia vitripennis]	19	60481	8.71	K.EVVANLNNFTDMIVEER.R
gi|157111245	cohesin-subunit, putative [Aedes aegypti]	19	190734	5.56	R.CEEMQEILSNVEEDNMLK.S
gi|115672685	PREDICTED: hypothetical protein, partial [Strongylocentrotus purpuratus]	19	10641	6.95	R.LSEISGGSMFIDK.K
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	19	47623	7.81	R.LLVASQPEVLI.-
gi|196005431	hypothetical protein TRIADDRAFT_25672 [Trichoplax adhaerens]	19	122620	6.47	R.YLLQNCAIEIFLANRR.S
gi|170577781	hypothetical protein Bm1_13350 [Brugia malayi]	18	36854	5.94	R.NQAVMSESMLKENDLLR.Y + Oxidation (M)
gi|242011174	zinc finger protein ush, putative [Pediculus humanus corporis]	18	115220	8.13	K.SSSSTISSSSSTSSSSNNK.T
gi|268553951	C. briggsae CBR-DIS-3 protein [Caenorhabditis briggsae]	18	117170	8.01	K.YPLGHYVRSIGEMGSR.E R.YAFSVIWTMTSNADIQSVKYHK.S

Appendix Table A10 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	18	194679	8.93	R.RVSEGNAPHSK.N
gi|115659	RecName: Full=Short-chain collagen C4	18	36445	4.58	K.GDTGAPGPQGPK.G
gi|91085763	PREDICTED: similar to CG11337 CG11337-PA [Tribolium castaneum]	18	83090	8.68	K.VPDLEFGGIYTATIVELR.D
gi|15425683	Kettin [Procambarus clarkii]	18	546739	5.58	R.IDWFRDGQPIQMGSR.F + Oxidation (M)
gi|242021545	hypothetical protein Phum_PHUM515400 [Pediculus humanus corporis]	17	180145	9.37	K.SLVEKSDPNAVPK.M
gi|268580207	C. briggsae CBR-UNC-18 protein [Caenorhabditis briggsae]	17	67776	6.7	K.LCKVEQDLSTGSDAEGER.V
gi|242005496	conserved hypothetical protein [Pediculus humanus corporis]	17	117349	5.6	K.LLPKSIEPVK.M
gi|76156474	SJCHGC03876 protein [Schistosoma japonicum]	17	23805	5.84	K.CHSDESCTCDIIDLLR.Q
gi|268568822	C. briggsae CBR-PQN-83 protein [Caenorhabditis briggsae]	17	40656	8.16	R.RATSGGGSNIGGGGGGNFGSR.G
gi|268562018	Hypothetical protein CBG12906 [Caenorhabditis briggsae]	17	35028	7.2	K.IDGSPIVVPVK.D
gi|157118786	hypothetical protein AaeL_AAEL008398 [Aedes aegypti]	17	263152	6.34	K.SSSGVMGTQRPMMHKR.L + 2 Oxidation (M)
gi|256070183	hypothetical protein [Schistosoma mansoni]	16	45798	7.19	K.KPLTRNKPVK.T
gi|157125153	hypothetical protein AaeL_AAEL010109 [Aedes aegypti]	16	20735	6.54	K.TISTVMNT.- + Oxidation (M)
gi|115699895	PREDICTED: similar to formin binding protein 11-related protein [Strongylocentrotus purpuratus]	16	140750	8.57	R.FNGMLGKPGSTPLDLFK.F
gi|158287207	AGAP011349-PA [Anopheles gambiae str. PEST]	16	65162	9.04	R.SERLPQQPK.V

Appendix Table A10 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	16	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|193671761	PREDICTED: similar to DNA-J [Acyrthosiphon pisum]	16	42116	8.89	M.EVNKDEAYR.C
gi|268562870	C. briggsae CBR-SEA-2 protein [Caenorhabditis briggsae]	16	253950	7.42	K.SRNPEYPKPPAK.R
gi|268552739	Hypothetical protein CBG17702 [Caenorhabditis briggsae]	16	55277	9.63	R.DSAITGEQINEDK.I
gi|157125808	membrane traffic protein [Aedes aegypti]	16	51628	8.84	R.MAALVCIPQTTSK.R

Appendix Table A11 Protein identification data form LCMS analysis. This table presented data of gel number 4 (G4) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|34500282	Bm95 protein [Rhipicephalus microplus]	72	67154	6.44	R.NAECEVVPGAEDDFVCK.C
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	32	194679	8.93	R.RVSEGNAPHSK.N
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	32	34737	8.06	K.IINLAQGKPK.G
gi|115659	RecName: Full=Short-chain collagen C4	28	36445	4.58	K.GDTGAPGPQGPK.G
gi|419953	glycoprotein antigen BM86 - southern cattle tick (fragments)	25	21100	6.05	K.ANCQCPPDTRPGEIGCIEK.D
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	24	174650	5.27	R.GAPGLSGLKGPK.G
gi|157137991	pcdc2/rp-8 (programmed cell death protein 2) [Aedes aegypti]	22	41222	4.87	R.KVNYCGVIHQR.I
gi|15298096	green fluorescent protein [Montastraea cavernosa]	22	26002	6.09	K.LRMQGVVNGHK.F
gi|156408279	predicted protein [Nematostella vectensis]	21	61638	9.65	R.CDSPKPSPSSSDNEDADATFNR.S
gi|156365595	predicted protein [Nematostella vectensis]	21	68246	6.45	R.EDPALGSEHK.T
gi|156546758	PREDICTED: similar to ATP-binding cassette sub-family A member 3, putative [Nasonia vitripennis]	20	187844	8.66	R.MPNEFYDVACK.S
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	20	58116	9.25	R.YHQKPLAPK.V
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	20	101408	5.79	K.KATEYGSTPK.W
gi|47169657	keyhole limpet hemocyanin2 [Megathura crenulata]	20	394427	5.74	K.KPYNAAK.C
gi|194245494	carbamoylphosphate synthetase [Scaphinotus petersi]	20	75451	5.57	K.EPYDLR.I

Appendix Table A11 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|189236109	PREDICTED: similar to nucleoporin 37 [Tribolium castaneum]	20	36446	5.08	K.LLIAEKIGLIR.F
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	19	44167	8.9	K.MPGPTVVPIR.E + Oxidation (M)
gi|268562225	Hypothetical protein CBG05575 [Caenorhabditis briggsae]	19	18215	4.75	K.VVRSPNKPGK.G
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	19	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|157117680	polybromo-1 [Aedes aegypti]	19	192825	6.14	K.NPIDLERIEFK.L
gi|91092588	PREDICTED: similar to CG12125 CG12125-PA [Tribolium castaneum]	19	42908	4.56	K.DFLIGYEEMLK.Y + Oxidation (M)
gi|242025626	hypothetical protein Phum_PHUM617340 [Pediculus humanus corporis]	19	24213	5.13	R.IDGIEARFEPVK.H
gi|157120630	hypothetical protein AaeL_AAEL009087 [Aedes aegypti]	19	31539	6.01	R.YHMVIHGPK.K
gi|156347792	hypothetical protein NEMVEDRAFT_v1g221608 [Nematostella vectensis]	19	18151	4.73	K.LNDDLALTNQTR.A
gi|158294294	AGAP005512-PA [Anopheles gambiae str. PEST]	18	35362	9.41	R.KPYDLR.K
gi|115655418	PREDICTED: similar to solute carrier family 16, member 10 [Strongylocentrotus purpuratus]	18	75701	7.53	R.EMMIPR.E + Oxidation (M)
gi|241708208	LIM domain-binding protein, putative [Ixodes scapularis]	17	60818	9.3	K.IPICSNCGSPIR.G
gi|242014445	kif1, putative [Pediculus humanus corporis]	17	192495	5.81	R.DLLNPNPKGHLR.V
gi|170047320	gp210 [Culex quinquefasciatus]	17	206844	5.86	R.NMIYENGPER.I + Oxidation (M)
gi|240953833	condensin-2 complex subunit H2, putative [Ixodes scapularis]	17	78660	4.85	R.EPYDIR.T

Appendix Table A11 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|221108679	PREDICTED: hypothetical protein, partial [Hydra magnipapillata]	17	6411	9.5	K.HSLSPLCLKHR.T
gi|270004586	hypothetical protein TcasGA2_TC003950 [Tribolium castaneum]	17	243035	9.47	K.KPYEVR.R
gi|170582999	Homeobox protein ceh-32 [Brugia malayi]	17	30353	9.59	K.KYPMPR.T
gi|61654660	L23a [Suberites domuncula]	17	17375	10.65	R.QPMYPR.R
gi|157135755	DEAD box ATP-dependent RNA helicase [Aedes aegypti]	16	82792	9.04	R.KPKQQNGGKPR.F
gi|256077887	hypothetical protein [Schistosoma mansoni]	16	60292	8.71	K.KTLPISFAEVSR.S
gi|156374956	predicted protein [Nematostella vectensis]	16	198499	5.29	R.IAAALLKGGLYEK.V
gi|115717773	PREDICTED: similar to EG:49E4.1 [Strongylocentrotus purpuratus]	16	494696	5.87	K.VNSYLGFGPK.A
gi|76157548	SJCHGC08753 protein [Schistosoma japonicum]	16	19538	6.36	K.ELIEYMPDMCLVRDLLAK.A + Oxidation (M)
gi|242007326	conserved hypothetical protein [Pediculus humanus corporis]	16	130065	8.43	K.DLVHEMVLK.K + Oxidation (M)
gi|84097160	cytochrome oxidase subunit 1 [Cephise sp. Burns01]	15	18151	5.26	R.INNMSFFQMPL.- + 2 Oxidation (M)
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	15	126171	7.14	R.IITLLRQIIR.T
gi|115749236	PREDICTED: similar to BIG3, partial [Strongylocentrotus purpuratus]	15	169014	5.89	K.MEAAHIKGIIR.A
gi|193709352	PREDICTED: similar to NFX1-type zinc finger-containing protein 1 [Acyrthosiphon pisum]	15	208997	8.32	K.MIHAAMSK.S
gi|76154409	SJCHGC07860 protein [Schistosoma japonicum]	15	14358	5.32	R.GPQYDGYGPPMG.-

Appendix Table A11 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|193620405	PREDICTED: similar to Tigger transposable element-derived protein 6, partial [Acyrthosiphon pisum]	15	49833	8.62	K.NDARINGPILR.Q
gi|118790579	AGAP009643-PA [Anopheles gambiae str. PEST]	14	124153	6.78	K.MIDMHVK.G + Oxidation (M)
gi|242007732	Nuclear transcription factor Y subunit alpha, putative [Pediculus humanus corporis]	14	44987	8	K.TPVQVKPTVR.L
gi|58381243	AGAP000053-PA [Anopheles gambiae str. PEST]	14	45055	5.25	R.CIAAMASRMQSADSASELR.D + Oxidation (M)
gi|241758359	protein wingless, putative [Ixodes scapularis]	14	36314	10.08	K.TCWKSLPAFSQVGAYLVQR.Y

Appendix Table A12 Protein identification data form LCMS analysis. This table presented data of gel number 4 (G4) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1468981	angiotensin-converting enzyme-like protein [Rhipicephalus microplus]	123	75495	5.84	K.DKDLPLEPDLTR.N K.LSNEAASLDGYDNIK.S
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34	34737	8.06	K.IINLAQGKPK.G
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	28	66947	8.64	K.ILKNLAGKPK.I
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	28	138255	6.59	R.GKLIQLQPK.T
gi|270009184	hypothetical protein TcasGA2_TC015840 [Tribolium castaneum]	25	95268	8.33	K.QDDIEIMTFRK.N + Oxidation (M)
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	25	112787	4.31	K.LETKGLFMSR.L
gi|221122805	PREDICTED: similar to predicted protein [Hydra magnipapillata]	25	110792	8.98	R.ERPTINKPKR.D
gi|13359096	Esterase-like protein (ESR-LP) [Bombyx mori]	25	34010	5.69	K.IWEELYPITN.-
gi|170042649	conserved hypothetical protein [Culex quinquefasciatus]	25	94398	9.36	R.NSLRDILQLIAR.T
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	24	48763	5.94	K.LGGEKPAKPK.E
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	24	60310	6.56	K.TYFKIQPK.L
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	24	125426	9.1	R.VGINRLKPK.L
gi|170586404	F-box domain containing protein [Brugia malayi]	24	53036	9.66	R.QNALLIQPK.A
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	24	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	24	150695	6.9	K.SCEYIKPK.E

Appendix Table A12 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|115903618	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	24	87637	5.44	K.SDIISHKPK.K
gi|255683281	alkaline phosphatase [Bombyx mori]	24	59626	5.82	MVVSCGGGGGGGGAGEGRGPLPPGAAR.A
gi|268556274	Hypothetical protein CBG01376 [Caenorhabditis briggsae]	23	91066	6.63	R.ENKSLSPLALIAR.K
gi|193617873	PREDICTED: similar to Multiple inositol polyphosphate phosphatase 1 CG4123-PA [Acyrthosiphon pisum]	23	54303	8.28	K.QNLSYENVSLMLESCMYEK.A
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	23	75672	9.35	K.TLTFEEIRK.K
gi|89145849	inositol trisphosphate receptor [Aplysia californica]	23	318416	5.61	K.DQDVRMNELMK.L + 2 Oxidation (M)
gi|91079338	PREDICTED: similar to AGAP009236-PA [Tribolium castaneum]	22	80386	5.74	R.DLDTLLSMEIK.L
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	22	78492	8.75	R.SPELLLQPK.T
gi|256073012	DNA-directed RNA polymerase I [Schistosoma mansoni]	22	201143	6.62	K.GGMVNAQQMSVALGQIELEGR.R + Oxidation (M)
gi|189236109	PREDICTED: similar to nucleoporin 37 [Tribolium castaneum]	22	36446	5.08	K.LLIAEKIGLIR.F
gi|115749236	PREDICTED: similar to BIG3, partial [Strongylocentrotus purpuratus]	22	169014	5.89	K.MEAAHIKGIIR.A
gi|256087746	hypothetical protein [Schistosoma mansoni]	21	136058	8.48	R.TNWSPSLDLVGHRK.H
gi|50841460	homeobox protein sine oculis six 1/2 [Leucosolenia eleanor]	21	4621	9.43	R.DLAEQTGLTTTQ.-
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	21	194679	8.93	R.RVSEGNAPHSK.N
gi|157106621	DNA polymerase eta [Aedes aegypti]	21	95165	7.49	R.KRPTSGDK.S

Appendix Table A12 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156400082	predicted protein [Nematostella vectensis]	21	62244	9.78	K.EDKLLDHR.D
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	21	58116	9.25	R.YHQKPLAPK.V
gi|268567117	Hypothetical protein CBG17912 [Caenorhabditis briggsae]	21	37983	9.16	R.QDIPMDHSTSHK.F + Oxidation (M)
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	21	174650	5.27	R.GAPGLSGLKGPK.G
gi|193660945	PREDICTED: similar to CG32795 CG32795-PB [Acyrthosiphon pisum]	20	41104	9.43	K.LVLSIIGFFLSLLNLVTHRR.G
gi|15298096	green fluorescent protein [Montastraea cavernosa]	20	26002	6.09	K.LRMQGVVNGHK.F
gi|242005170	alpha-taxilin, putative [Pediculus humanus corporis]	20	59381	5.89	R.DENIEMQNKFK.T + Oxidation (M)
gi|156362206	predicted protein [Nematostella vectensis]	20	34377	5.63	R.TPGSEGHRIVK.T
gi|170587102	GRIP domain containing protein [Brugia malayi]	20	52617	5.19	K.GLEEEMERLR.R + Oxidation (M)
gi|170033889	conserved hypothetical protein [Culex quinquefasciatus]	20	97092	7.49	K.TAKQLEYTAPR.T
gi|238654492	muscular protein 20 [Tentyria rotundata]	20	17281	5.47	K.MMENINSFQAAIK.A + Oxidation (M)
gi|260207803	muscular protein 20 [Pimelia subglobosa]	20	17197	5.52	K.MMENINSFQAALK.T + Oxidation (M)
gi|156383423	predicted protein [Nematostella vectensis]	20	11177	5.05	R.SIDDFWTGVP.-
gi|242016348	hypothetical protein Phum_PHUM400690 [Pediculus humanus corporis]	20	40849	9.6	K.SDNLRGSIGDFMNLK.R
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	20	115140	8.72	K.MGPPMQGR.G + Oxidation (M)

Appendix Table A12 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170047320	gp210 [Culex quinquefasciatus]	20	206844	5.86	R.NMIYENGPER.I + Oxidation (M)
gi|193700019	PREDICTED: hypothetical protein [Acyrthosiphon pisum]	19	83753	4.73	K.SEVLSSNNVAQSSNAEIILTK.T
gi|156366213	predicted protein [Nematostella vectensis]	19	56054	8.98	K.GAAGSRPPSPER.D
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	19	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|115634798	PREDICTED: similar to ENSANGP00000007239 [Strongylocentrotus purpuratus]	19	305747	5.9	R.EKHDSSPGILR.Q

Appendix Table 13 Protein identification data form LCMS analysis. This table presented data of gel number 5 (G5) which found protein bands in tick’s gut proteins.

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268558454	Hypothetical protein CBG18883 [Caenorhabditis briggsae]	34	302891	4.66	R.CEIFNDVEKVETACIVER.I
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	26	194679	8.93	R.RVSEGNAPHSK.N
gi|3426184	SEVENLESS [Anopheles sp.]	24	99503	8.47	R.CQNTSEDTQIIAPFPATCSK.V
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	24	20283	7.79	R.GNGGGGGGGGGSR.G
gi|242005381	synaptic vesicular amine transporter, putative [Pediculus humanus corporis]	23	51848	8.54	K.TIFDVPNMENGRMSILLSSK.A + Oxidation (M)
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	21	126171	7.14	R.IITLLRQIIR.T
gi|115927439	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	21	94252	8.53	K.SFARFTKPER.D
gi|193590624	PREDICTED: similar to Breast cancer metastasis-suppressor 1-like protein (BRMS1-homolog protein p40) (BRMS1-like protein p40) [Acyrthosiphon pisum]	21	32877	5.81	R.RPYGHVQPKR.K
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	20	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|158518877	cytochrome oxidase subunit I [Pheidole rhea]	20	38418	6.01	-.LMLHFAIGAGMIGSSMSMIIR.L + 2 Oxidation (M)
gi|242013855	conserved hypothetical protein [Pediculus humanus corporis]	20	376680	5.3	K.CENMLAMKDETELNLNNR.I + Oxidation (M)
gi|195996053	hypothetical protein TRIADDRAFT_51865 [Trichoplax adhaerens]	19	147604	4.94	K.STHDVAVKLLR.D
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	19	58245	8.67	K.VRYYQTPVR.D
gi|268552205	Hypothetical protein CBG01631 [Caenorhabditis briggsae]	18	130900	6.38	K.GPVTTYWLLGKQSESQMQQK.N + Oxidation (M)
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	18	52382	4.11	K.NGGGDDDDDDDEGNGFGRGGGGR.G

Appendix Table A13 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|15298096	green fluorescent protein [Montastraea cavernosa]	18	26002	6.09	K.LRMQGVVNGHK.F
gi|115753253	PREDICTED: hypothetical protein, partial [Strongylocentrotus purpuratus]	18	54756	7.95	K.LLSSVKWYSR.E
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	17	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|262401361	hypothetical protein [Scylla paramamosain]	17	5011	10.23	M.AVGGEVGGGSR.R
gi|256070183	hypothetical protein [Schistosoma mansoni]	17	45798	7.19	K.KPLTRNKPVK.T
gi|189236109	PREDICTED: similar to nucleoporin 37 [Tribolium castaneum]	16	36446	5.08	K.LLIAEKIGLIR.F
gi|196004921	hypothetical protein TRIADDRAFT_56227 [Trichoplax adhaerens]	16	15043	7.07	K.LLVEHGGDVNK.G
gi|157361577	astacin-like zinc metalloprotease-like protein [Phlebotomus papatasi]	16	30635	5.28	K.SGNFEGDMILSPRQMIDLR.F + 2 Oxidation (M)
gi|91082899	PREDICTED: similar to spire CG10076-PB [Tribolium castaneum]	15	80630	6.98	R.APIEYELTPYEILMKDIR.S + Oxidation (M)
gi|66509122	PREDICTED: similar to CG11594-PA, isoform A isoform 1 [Apis mellifera]	15	60540	6.4	K.AMGGSATMVKPR.S + 2 Oxidation (M)
gi|170583347	hypothetical protein Bm1_25390 [Brugia malayi]	15	22336	9.18	K.TPVTPKQIVLK.N
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	15	112787	4.31	K.LETKGLFMSR.L
gi|193697476	PREDICTED: similar to rCG49027 [Acyrthosiphon pisum]	15	38602	8.82	K.EEFSIAVGTNQGSVQIFDLR.A
gi|170047320	gp210 [Culex quinquefasciatus]	15	206844	5.86	R.NMIYENGPER.I + Oxidation (M)
gi|170035285	conserved hypothetical protein [Culex quinquefasciatus]	14	43148	7.85	K.VQAQNAHNGAVSGSAAIDTENR.F

Appendix Table A13 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	14	47623	7.81	R.LLVASQPEVLI.-
gi|110757480	PREDICTED: hypothetical protein [Apis mellifera]	14	59598	6.46	R.SSLDQMIQQTMDDEESPSAR.S
gi|193627330	PREDICTED: similar to leucine-rich transmembrane protein [Acyrthosiphon pisum]	14	82014	6.41	R.GNYLDFLSEHSFDGILNLR.T
gi|170047092	serrate protein [Culex quinquefasciatus]	14	419035	5.51	R.IRLGCTAR.Y
gi|158295216	AGAP006039-PA [Anopheles gambiae str. PEST]	14	47702	5.87	R.GGGGRESPLLIPPTYAAASLSLVQPLLK.R
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	14	48812	8.57	K.SQKYGHVK.S
gi|91079981	PREDICTED: similar to AGAP005961-PA [Tribolium castaneum]	14	91409	6.59	K.GTGKQLHDAVR.V
gi|221127865	PREDICTED: similar to MGC84609 protein [Hydra magnipapillata]	14	30318	5.97	K.NEGLLNVCRISLHLK.Y
gi|211938970	Chain A, A Non-Cytotoxic Dsred Variant For Whole-Cell Labeling	13	24965	7.28	K.SIYMAKKPVK.L + Oxidation (M)
gi|157135755	DEAD box ATP-dependent RNA helicase [Aedes aegypti]	13	82792	9.04	R.KPKQQNGGKPR.F
gi|115712231	PREDICTED: similar to LOC495952 protein, partial [Strongylocentrotus purpuratus]	13	41446	6.24	R.LDGKGQGGSK.G
gi|156543856	PREDICTED: similar to CG33715-PD [Nasonia vitripennis]	13	876467	5.36	K.SSFESIFGQPLKTPEAETSLK.L
gi|195995739	hypothetical protein TRIADDRAFT_51542 [Trichoplax adhaerens]	13	54215	9.36	-.MASLIFSMDNRDHAYIVIR.Q + Oxidation (M)
gi|157106919	hypothetical protein AaeL_AAEL014757 [Aedes aegypti]	13	30902	8.87	R.RSNETHGSRPSKPR.S
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	13	10796	6.08	K.GVVGTIVVNGEGIPIK.S

Appendix Table A13 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|3660698	period [Cydia pomonella]	13	39618	5.77	R.ILNEVPTRPAELVKQQMTK.R + Oxidation (M)
gi|156384835	predicted protein [Nematostella vectensis]	13	18421	9.65	K.SGAKQSPFFR.K
gi|156393510	predicted protein [Nematostella vectensis]	13	14503	10.08	R.NKTQDIIHR.T
gi|193702309	PREDICTED: similar to GA18954-PA [Acyrthosiphon pisum]	13	66125	8.63	-.MIHSWSGK.T
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	13	110364	5.32	K.MNPFNVPK.T

Appendix Table A14 Protein identification data form LCMS analysis. This table presented data of gel number 5 (G5) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	31	85695	7.94	K.AATPSPKQPK.E
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	28	24881	9.76	K.RADPGIFAWEIR.D
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	27	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|256073417	hypothetical protein [Schistosoma mansoni]	25	51173	9.54	R.LVQRLDSMISLR.R
gi|15298096	green fluorescent protein [Montastraea cavernosa]	23	26002	6.09	K.LRMQGVVNGHK.F
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	23	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22	22842	5.43	M.ASLTIPKAPK.K
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	22	40519	6.4	K.EGGPPKSKPK.K
gi|170589507	Raf kinase [Brugia malayi]	22	83798	9.03	R.DLKTNNIFLTDDGTVK.I
gi|156380891	predicted protein [Nematostella vectensis]	21	31522	10.15	K.IHMPPGSK.V + Oxidation (M)
gi|91088841	PREDICTED: similar to enoki mushroom CG11290-PA [Tribolium castaneum]	21	268909	9.19	K.SSDMDIEKIDDDR.N + Oxidation (M)
gi|196009101	hypothetical protein TRIADDRAFT_36227 [Trichoplax adhaerens]	21	52031	6.41	K.GLVDLLNTK.V
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|170586404	F-box domain containing protein [Brugia malayi]	21	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T

Appendix Table A14 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|170048915	conserved hypothetical protein [Culex quinquefasciatus]	20	46121	7	R.DALQMSSEMVLLDLLASWSR.E + Oxidation (M)
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	20	9092	9.51	-.MECLRSVLTIAGK.A
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	19	56983	9.9	-.TLTHRVR.L
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	19	65748	9.67	R.TITHSPVK.S
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	19	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	19	52382	4.11	K.NGGGDDDDDDDEGNGFGRGGGGGR.G
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	19	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|76154409	SJCHGC07860 protein [Schistosoma japonicum]	18	14358	5.32	R.GPQYDGYGPPMG.-
gi|115893528	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	18	107102	8.71	K.DILPFDHSR.Y
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	18	194679	8.93	R.RVSEGNAPHSK.N
gi|4218957	cytoplasmic polyadenylation element-binding protein [Spisula solidissima]	18	74011	8.13	R.NTKGHTNLALL.-
gi|158291121	AGAP002345-PA [Anopheles gambiae str. PEST]	17	87465	5.38	R.QCGAIFPHHENMSLLQQQK.L + Oxidation (M)
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	17	16163	9.54	-.MAALLLQLNSCSR.K

Appendix Table A14 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|29840983	hypothetical protein [Schistosoma japonicum]	17	36215	5.83	K.TLTHGKPK.C
gi|72011097	PREDICTED: similar to NPAS3 (MOP6) [Strongylocentrotus purpuratus]	17	103564	8.13	K.RGTHAIAR.E
gi|268576849	Hypothetical protein CBG16029 [Caenorhabditis briggsae]	17	30164	5.37	K.NGSAEIEFLVEIDTEK.S
gi|156549170	PREDICTED: similar to ENSANGP00000016875 [Nasonia vitripennis]	17	207954	5.1	K.LSQEYKSVELSLNQR.V
gi|262305467	protein kinase [Aphonopelma chalcodes]	17	34487	8.26	R.EVPFSDLSPMEIGMKIATEGLR.I + 2 Oxidation (M)
gi|115623529	PREDICTED: similar to ubiquitin-activating enzyme E1-like 2 [Strongylocentrotus purpuratus]	17	146009	5.09	K.LSQQTLADNSDLTFLK.Q
gi|170583347	hypothetical protein Bm1_25390 [Brugia malayi]	17	22336	9.18	K.TPVTPKQIVLK.N
gi|256071347	hypothetical protein [Schistosoma mansoni]	17	12954	8.84	K.LNTKEDNEDLAMCPK.R + Oxidation (M)
gi|156368134	predicted protein [Nematostella vectensis]	17	82312	5.78	K.EAQEDLETLLNMEMK.L
gi|156366213	predicted protein [Nematostella vectensis]	16	56054	8.98	K.GAAGSRPPSPER.D
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	16	138300	5.84	K.LMVKYDDIK.I
gi|193662148	PREDICTED: similar to NFX1-type zinc finger-containing protein 1 [Acyrthosiphon pisum]	16	314032	7.3	K.WMNQNDEEICLKQCPLCK.T + Oxidation (M) K.DPSEIVFVMSNEVNGFMDLFK.Q + Oxidation (M)
gi|157128573	hypothetical protein AaeL_AAEL002392 [Aedes aegypti]	16	227443	6.97	K.TKETNLEKPQQTYSK.S
gi|221119725	PREDICTED: similar to predicted protein [Hydra magnipapillata]	16	1044655	5.5	R.VAVNSIKNK.L
gi|121256	RecName: Full=Globin CTT-IIIA	16	16144	6.11	K.ALLNQLGTSHK.A

Appendix Table A14 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268536686	Hypothetical protein CBG06249 [Caenorhabditis briggsae]	16	70367	8.93	K.DLDDAITSDMEQGGRR.A + Oxidation (M)
gi|157136515	hypothetical protein AaeL_AAEL003489 [Aedes aegypti]	15	85543	9.15	R.TISQMFR.D
gi|158291101	AGAP002353-PA [Anopheles gambiae str. PEST]	15	46543	6.08	K.IIQMNALAPVAFMGHMKSPLLR.F + Oxidation (M)
gi|72048496	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	15	56261	8.39	R.HLRSLTEATMMESVDPTYAR.T + 2 Oxidation (M)
gi|30515682	coproporphyrinogen oxidase [Aplysia californica]	15	45050	8.46	K.DVVHFHK.T

Appendix Table A15 Protein identification data form LCMS analysis. This table presented data of gel number 5 (G5) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|115659	RecName: Full=Short-chain collagen C4	32	36445	4.58	K.GDTGAPGPQGPK.G
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	27	75672	9.35	K.TLTFEEIRK.K
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	27	66947	8.64	K.ILKNLAGKPK.I
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	26	112787	4.31	K.LETKGLFMSR.L
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	24	20283	7.79	R.GNGGGGGGGGGSR.G
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	24	194679	8.93	R.RVSEGNAPHSK.N
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	23	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|196006884	hypothetical protein TRIADDRAFT_7238 [Trichoplax adhaerens]	23	15711	9.38	K.TAAKGSPFFR.K
gi|91081233	PREDICTED: similar to hormone-sensitive lipase [Tribolium castaneum]	23	84776	5.09	K.YKSKPPAR.G
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	23	9092	9.51	-.MECLRSVLTIAGK.A
gi|268580415	C. briggsae CBR-PPK-3 protein [Caenorhabditis briggsae]	22	170200	5.58	R.EVDGTFMKVR.E
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	22	40519	6.4	K.EGGPPKSKPK.K
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	22	99637	4.8	K.ALLNGLKAEPR.V
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	22	126171	7.14	R.IITLLRQIIR.T
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	22	34737	8.06	K.IINLAQGKPK.G

Appendix Table A15 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	22	23318	10.58	K.ENQKPIKPK.T
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	21	60310	6.56	K.TYFKIQPK.L
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	21	125426	9.1	R.VGINRLKPK.L
gi|170586404	F-box domain containing protein [Brugia malayi]	21	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	21	150695	6.9	K.SCEYIKPK.E
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	21	48812	8.57	K.SQKYGHVK.S
gi|170580994	U1 zinc finger family protein [Brugia malayi]	20	51370	7.07	K.IDGPIEFR.K
gi|268559272	Hypothetical protein CBG19372 [Caenorhabditis briggsae]	20	151096	8.99	R.SVSIASNSQSQLQK.M
gi|158297905	AGAP004765-PA [Anopheles gambiae str. PEST]	20	82488	6.3	K.MLKGEYTLK.A
gi|156401368	predicted protein [Nematostella vectensis]	20	55046	6.23	K.DISQTLANFK.K
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	19	78492	8.75	R.SPELLLQPK.T
gi|118781575	AGAP010405-PA [Anopheles gambiae str. PEST]	19	17503	9.32	K.TAQSMMRPK.S + 2 Oxidation (M)

Appendix Table A15 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|91079981	PREDICTED: similar to AGAP005961-PA [Tribolium castaneum]	19	91409	6.59	K.GTGKQLHDAVR.V
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	19	16163	9.54	-.MAALLLQLNSCSR.K
gi|72167364	PREDICTED: similar to aquaporin 4, partial [Strongylocentrotus purpuratus]	18	33778	4.77	K.DDLGSADFWR.A
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|289739695	vesicle-associated membrane protein-associated protein A [Glossina morsitans morsitans]	18	28271	8.3	K.TETIAPPKPK.A
gi|156543266	PREDICTED: similar to conserved hypothetical protein [Nasonia vitripennis]	18	39769	5.66	R.SRQLQMYR.V
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	18	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|158302538	AGAP001106-PA [Anopheles gambiae str. PEST]	18	118904	6.3	R.QRPGSADSK.K
gi|241561644	synembryn, putative [Ixodes scapularis]	17	14334	4.73	K.LARGGVVQPAR.V
gi|241654886	lipid exporter ABCA1, putative [Ixodes scapularis]	17	23912	7.98	K.AFPKTLSGGMK.Q
gi|170047092	serrate protein [Culex quinquefasciatus]	17	419035	5.51	R.IRLGCTAR.Y
gi|195998982	hypothetical protein TRIADDRAFT_21641 [Trichoplax adhaerens]	17	74731	8.71	K.KGDVVALYMESCPEFVMIWLGLSK.L + 2 Oxidation (M)
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	17	58245	8.67	K.VRYYQTPVR.D
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	17	174650	5.27	R.GAPGLSGLKGPK.G
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	16	110364	5.32	K.MNPFNVPK.T

Appendix Table A15 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156340946	hypothetical protein NEMVEDRAFT_v1g147631 [Nematostella vectensis]	16	41345	4.83	K.LLVIVSRPAR.L
gi|242024928	hypothetical protein Phum_PHUM603300 [Pediculus humanus corporis]	16	295267	9.04	K.VDYTILEKR.E
gi|169119261	Rel2 [Anopheles arabiensis]	16	25443	6.17	R.THGSLMGSR.T
gi|193624948	PREDICTED: similar to gag-like protein [Acyrthosiphon pisum]	16	48472	8.8	R.STLVKEEASFK.R
gi|156392182	predicted protein [Nematostella vectensis]	16	57541	9.23	R.GSAPPPPPAR.M

Appendix Table A16 Protein identification data form LCMS analysis. This table presented data of gel number 6 (G6) which found protein bands in tick’s gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	33	112787	4.31	K.LETKGLFMSR.L
gi|256078034	initiation factor 5a [Schistosoma mansoni]	31	17855	5.41	K.DYQLVDIK.D
gi|195998638	hypothetical protein TRIADDRAFT_21077 [Trichoplax adhaerens]	29	36004	9.91	R.DYNNKVIK.K
gi|170592699	ABC transporter family protein [Brugia malayi]	29	253101	8.2	R.DNYLIDLK.Y
gi|157107880	hypothetical protein AaeL_AAEL014908 [Aedes aegypti]	27	45804	4.84	K.MNMILINK.S + Oxidation (M)
gi|158297636	AGAP011471-PA [Anopheles gambiae str. PEST]	27	70551	6.66	K.MNMILLNK.S + Oxidation (M)
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	27	23318	10.58	K.ENQKPIKPK.T
gi|270006505	odorant receptor 278 [Tribolium castaneum]	27	45162	9.06	K.NDYLKVLK.L
gi|91081349	PREDICTED: similar to aspartyl-tRNA synthetase [Tribolium castaneum]	27	51577	6.84	-.MFINILNK.N
gi|221115220	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	27	37928	6.17	K.NNFLEEVK.N
gi|241694276	conserved hypothetical protein [Ixodes scapularis]	26	34468	8.14	R.GGYLVKDLK.S
gi|242025562	carnitine O-palmitoyltransferase 2, putative [Pediculus humanus corporis]	25	68767	7.6	K.NELLNMMK.H
gi|156544750	PREDICTED: similar to ribonuclease [Nasonia vitripennis]	25	110076	7.75	R.LQGIILPPVKMEQD.- + Oxidation (M) K.MAQEQSNALYFTYMLDLR.G + Oxidation (M)
gi|3183022	RecName: Full=50 kDa hatching enzyme; Short=HEZ; Short=HE; AltName: Full=Envelysin; AltName: Full=Sea-urchin-hatching proteinase; Contains: RecName: Full=38 kDa hatching enzyme; Contains: RecName: Full=32 kDa hatching enzyme non-specific; Contains:	24	66485	5.24	R.DDIAGITSLYGR.N
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	24	194679	8.93	R.RVSEGNAPHSK.N

Appendix Table A16 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	24	75672	9.35	K.TLTFEEIRK.K
gi|158287207	AGAP011349-PA [Anopheles gambiae str. PEST]	24	65162	9.04	R.SERLPQQPK.V
gi|157106621	DNA polymerase eta [Aedes aegypti]	22	95165	7.49	R.KRPTSGDK.S
gi|156363128	predicted protein [Nematostella vectensis]	22	37562	6.31	K.FPVRPLIIK.T
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	22	165556	6.1	K.MVLPRLQIIR.G
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	22	67527	9.56	K.AQINVALKIIR.N
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	21	66947	8.64	K.ILKNLAGKPK.I
gi|268578537	Hypothetical protein CBG13997 [Caenorhabditis briggsae]	21	25220	9.59	K.LLKIEQLLL.-
gi|115679221	PREDICTED: similar to DEAH (Asp-Glu-Ala-His) box polypeptide 38, partial [Strongylocentrotus purpuratus]	21	137807	6.38	K.QPEPVIPVKDNTSDMAQIAR.K + Oxidation (M)
gi|268580415	C. briggsae CBR-PPK-3 protein [Caenorhabditis briggsae]	20	170200	5.58	R.EVDGTFMKVR.E
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	19	34737	8.06	K.IINLAQGKPK.G
gi|170580994	U1 zinc finger family protein [Brugia malayi]	19	51370	7.07	K.IDGPIEFR.K
gi|156363765	predicted protein [Nematostella vectensis]	19	60740	5.76	K.IGEVVGAPGSEGTAASSSNKTMYK.G + Oxidation (M)
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	19	52382	4.11	K.NGGGDDDDDDDEGNGFGRGGGGGR.G
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	19	121129	8.33	K.GGGGGGGGGGGGDK.K

Appendix Table A16 (Continued)

Accession number	**Protein Hits**	**Protein score**	**Mw**	**PI**	**Peptide sequence**
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	19	56983	9.9	-.TLTHRVR.L
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	19	65748	9.67	R.TITHSPVK.S
gi|221130433	PREDICTED: similar to thioredoxin domain containing 11 [Hydra magnipapillata]	19	61790	9.12	R.LINRVNKPK.I
gi|3892088	prophenoloxidase [Anopheles gambiae]	19	78684	6.3	K.FTVTLNAGANTIVR.R
gi|91079981	PREDICTED: similar to AGAP005961-PA [Tribolium castaneum]	19	91409	6.59	K.GTGKQLHDAVR.V
gi|37703935	elongation factor-2 [Eurytemora affinis]	19	79396	5.78	K.LTTXXGKXVK.D
gi|289741131	OTU-like cysteine protease [Glossina morsitans morsitans]	18	37743	4.72	K.IDNEGSEMMR.H
gi|76155338	SJCHGC05221 protein [Schistosoma japonicum]	18	37634	5.89	-.PFQDEYKR.F
gi|115896473	PREDICTED: similar to MGC83212 protein [Strongylocentrotus purpuratus]	18	49626	10.28	K.LMKQDEYR.S
gi|72167364	PREDICTED: similar to aquaporin 4, partial [Strongylocentrotus purpuratus]	18	33778	4.77	K.DDLGSADFWR.A
gi|170582159	MSP domain protein VAP [Brugia malayi]	18	25339	6.34	K.SACGGYAANMDVEVRR.V / R.MSPESKSAHGGYAANMDVEVR.R + 2 Oxidation (M)
gi|15298096	green fluorescent protein [Montastraea cavernosa]	18	26002	6.09	K.LRMQGVVNGHK.F
gi|157125153	hypothetical protein AaeL_AAEL010109 [Aedes aegypti]	18	20735	6.54	K.TISTVMNT.- + Oxidation (M)
gi|18307516	putative venom metalloprotease [Pimpla hypochondriaca]	17	61759	6.79	R.CTHSRPVGVPK.G
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	17	20283	7.79	R.GNGGGGGGGGGSR.G

Appendix Table A16 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|242017225	DNA polymerase epsilon, catalytic subunit A, putative [Pediculus humanus corporis]	17	261671	6.5	K.LIDLKNNPVR.N
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	17	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|118779655	AGAP004060-PA [Anopheles gambiae str. PEST]	17	26601	4.57	R.LATLLSGRHVVSNYLKPEFTK.R
gi|115752581	PREDICTED: similar to FERM domain containing 4A [Strongylocentrotus purpuratus]	17	127891	9.37	R.RVELLVQPK.L
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	17	165126	9.32	R.ILLDQRVGAPR.Y

Appendix Table A17 Protein identification data form LCMS analysis. This table presented data of gel number 6 (G6) which found protein bands in Bm95 proteins.

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170592699	ABC transporter family protein [Brugia malayi]	37	253101	8.2	R.DNYLIDLK.Y
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	35	122944	8.75	K.VKGQSKPK.G / R.DVKILMGMNK.D + 2 Oxidation (M)
gi|268556740	Hypothetical protein CBG08656 [Caenorhabditis briggsae]	32	46743	5.75	-.DQIKMASEWGYNQEDIIAALK.E + Oxidation (M)
gi|242023447	conserved hypothetical protein [Pediculus humanus corporis]	31	48829	8.68	K.DFIENSLR.K
gi|268572719	Hypothetical protein CBG13254 [Caenorhabditis briggsae]	31	55921	6.59	K.NLMEITQK.F + Oxidation (M)
gi|256078034	initiation factor 5a [Schistosoma mansoni]	30	17855	5.41	K.DYQLVDIK.D
gi|268578419	C. briggsae CBR-NUCB-1 protein [Caenorhabditis briggsae]	30	52775	4.97	K.NMNEEDIK.A
gi|158294544	AGAP005651-PA [Anopheles gambiae str. PEST]	30	47687	5.78	R.NIFEDLNK.-
gi|242021826	Angiotensin-converting enzyme precursor, putative [Pediculus humanus corporis]	30	81610	9.34	K.NYILLSNR.A
gi|196015769	hypothetical protein TRIADDRAFT_33038 [Trichoplax adhaerens]	30	479542	5.78	K.LNFENDLK.I
gi|189233623	PREDICTED: similar to fibroblast growth factor receptor [Tribolium castaneum]	29	109725	6.63	K.MIDIETVR.A + Oxidation (M)
gi|193652752	PREDICTED: similar to exosome complex exonuclease RRP44 [Acyrthosiphon pisum]	29	94487	6.53	M.DFLENLSR.Q
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	28	52382	4.11	K.NGGGDDDDDDDEGNGFGRGGGGGR.G
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	26	75672	9.35	K.TLTFEEIRK.K
gi|158285848	AGAP007335-PA [Anopheles gambiae str. PEST]	25	121740	6.04	K.VQLMASEK.K K.MENCHSMRDAGGGGGGGGGGGMSVK.E + Oxidation (M)

Appendix Table A17 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268529728	C. briggsae CBR-HDA-2 protein [Caenorhabditis briggsae]	25	57705	5.4	K.NLNMPGIP.- + Oxidation (M) K.SLGKPLMVLGGGGYTLR.N + Oxidation (M)
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	24	112787	4.31	K.LETKGLFMSR.L
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	23	174650	5.27	R.GAPGLSGLKGPK.G
gi|29838553	budhead [Hydra vulgaris]	23	36936	7.27	K.VPRSPDKPGK.G
gi|268562225	Hypothetical protein CBG05575 [Caenorhabditis briggsae]	22	18215	4.75	K.VVRSPNKPGK.G
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	22	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	22	194679	8.93	R.RVSEGNAPHSK.N
gi|195999698	predicted protein [Trichoplax adhaerens]	21	58417	8.96	R.NVTEYLLAR.L
gi|15298096	green fluorescent protein [Montastraea cavernosa]	21	26002	6.09	K.LRMQGVVNGHK.F
gi|34597030	RNA polymerase II largest subunit [Ballophilus australiae]	21	10360	4.97	R.FQASQVQP.-
gi|242003806	conserved hypothetical protein [Pediculus humanus corporis]	20	86129	5.61	R.EKYCPLVTNGEGMELLEELAK.A + Oxidation (M)
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20	20283	7.79	R.GNGGGGGGGGGSR.G
gi|55977184	ommochrome binding protein 2 [Ostrinia nubilalis]	20	29528	6.37	K.VEGNAVRK.F
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	20	159499	5.87	R.DVTETLSTDV.-
gi|268554292	C. briggsae CBR-MYO-5 protein [Caenorhabditis briggsae]	19	219524	5.96	R.AAATETFRLR.N R.KVEGDLQHLQSEVEEALSDAK.V

Appendix Table A17 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|256073891	hypothetical protein [Schistosoma mansoni]	19	61705	8.85	K.GNLDTSVSQTSISTASRSPSSK.L
gi|156383423	predicted protein [Nematostella vectensis]	19	11177	5.05	R.SIDDFWTGVP.-
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	19	67527	9.56	K.AQINVALKIIR.N
gi|186973149	orcokinin precursor 1 [Dermacentor variabilis]	19	18254	9.19	R.LNGGELLR.S
gi|241293344	conserved hypothetical protein [Ixodes scapularis]	18	80487	9.07	R.EPNVVSQTAHSVARR.G
gi|283462204	celsr-like protein [Saccoglossus kowalevskii]	18	68159	4.4	K.VQGVNKAR.A
gi|110759515	PREDICTED: hypothetical protein [Apis mellifera]	18	21858	7.66	K.VQTLSKVP.-
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	18	34737	8.06	K.IINLAQGKPK.G
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	18	101408	5.79	K.KATEYGSTPK.W
gi|158284937	AGAP002199-PA [Anopheles gambiae str. PEST]	18	104013	9.64	R.IDAMFDDTR.S + Oxidation (M)
gi|118781274	Toll-like receptor (AGAP010669-PA) [Anopheles gambiae str. PEST]	18	116352	6.2	K.DVQTTDNMR.M
gi|221122039	PREDICTED: similar to TNF-receptor-associated factor 1 [Hydra magnipapillata]	18	44059	6.6	R.EPVLTLCGHR.L
gi|37703935	elongation factor-2 [Eurytemora affinis]	18	79396	5.78	K.LTTXXGKXVK.D
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	18	58116	9.25	R.YHQKPLAPK.V
gi|156547383	PREDICTED: similar to mCG141618 [Nasonia vitripennis]	17	465628	5.46	K.DIGVLIDK.I

Appendix Table A17 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	17	48812	8.57	K.SQKYGHVK.S
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	17	121129	8.33	K.GGGGGGGGGGGDK.K
gi|158293448	AGAP008690-PA [Anopheles gambiae str. PEST]	17	63872	9.59	K.VLLLNGAFQDGRK.L
gi|158451555	putative dopa decarboxylase protein [Rhescyntis hippodamia]	17	48620	6.16	R.LNGWILR.E
gi|170047092	serrate protein [Culex quinquefasciatus]	17	419035	5.51	R.IRLGCTAR.Y

Appendix Table A18 Protein identification data form LCMS analysis. This table presented data of gel number 6 (G6) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	31	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	31	60310	6.56	K.TYFKIQPK.L
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	31	125426	9.1	R.VGINRLKPK.L
gi|170586404	F-box domain containing protein [Brugia malayi]	31	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	31	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	31	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	31	150695	6.9	K.SCEYIKPK.E
gi|56756192	SJCHGC09241 protein [Schistosoma japonicum]	31	49876	5.38	K.VENGPRKPK.E
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	29	78492	8.75	R.SPELLLQPK.T
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	28	85695	7.94	K.AATPSPKQPK.E
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	25	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	24	34876	7.64	R.RGTDNAAVFK.F
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	23	20283	7.79	R.GNGGGGGGGGGSR.G
gi|193603792	PREDICTED: similar to glucosyl/glucuronosyl transferases, partial [Acyrthosiphon pisum]	23	27675	7.9	R.VNTFFHSVK.A
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	22	121129	8.33	K.GGGGGGGGGGGGDK.K

Appendix Table A18 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|242007128	conserved hypothetical protein [Pediculus humanus corporis]	21	50559	6.62	R.VDNDVAGYVK.S
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	21	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	21	194679	8.93	R.RVSEGNAPHSK.N
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	20	165126	9.32	R.ILLDQRVGAPR.Y
gi|196016867	predicted protein [Trichoplax adhaerens]	19	32742	7.55	K.NQEILKLVTR.S
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	19	115140	8.72	K.MGPPMQGR.G + Oxidation (M)
gi|170036073	Odorant receptor 92a [Culex quinquefasciatus]	19	46238	8.7	R.ITTTVRMIK.Y + Oxidation (M)
gi|156363128	predicted protein [Nematostella vectensis]	19	37562	6.31	K.FPVRPLIIK.T
gi|157135755	DEAD box ATP-dependent RNA helicase [Aedes aegypti]	19	82792	9.04	R.KPKQQNGGKPR.F
gi|170029058	conserved hypothetical protein [Culex quinquefasciatus]	18	26108	9.27	M.ELTPEVIRTR.W / R.RHCDGVMPQGPRPK.R + Oxidation (M)
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	18	24881	9.76	K.RADPGIFAWEIR.D
gi|196012718	hypothetical protein TRIADDRAFT_60295 [Trichoplax adhaerens]	18	78546	6.06	R.LLDTTTTDAK.E
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	17	6635	9.81	K.TITDNKEMK.T
gi|256073417	hypothetical protein [Schistosoma mansoni]	17	51173	9.54	R.LVQRLDSMISLR.R
gi|156362269	predicted protein [Nematostella vectensis]	17	173100	5.17	K.LEELLVLEKK.K

Appendix Table A18 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|241561644	synembryn, putative [Ixodes scapularis]	16	14334	4.73	K.LARGGVVQPAR.V
gi|157111532	f-box protein [Aedes aegypti]	16	67264	9.67	R.NDVSPALEVAPITPK.R
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	16	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	16	48812	8.57	K.SQKYGHVK.S
gi|196013067	hypothetical protein TRIADDRAFT_60463 [Trichoplax adhaerens]	16	87636	8.97	R.NVVQLLPPAK.F
gi|156540530	PREDICTED: hypothetical protein [Nasonia vitripennis]	16	35168	4.34	K.VSPAEQLLPKR.Q
gi|193647945	PREDICTED: similar to conserved hypothetical protein [Acyrthosiphon pisum]	16	145733	5.11	R.NQLELVNDLR.S
gi|156362206	predicted protein [Nematostella vectensis]	15	34377	5.63	R.TPGSEGHRIVK.T
gi|91076832	PREDICTED: similar to GA20540-PA [Tribolium castaneum]	15	145934	7.49	R.VQSEFDQVK.L
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	15	112787	4.31	K.LETKGLFMSR.L
gi|241162965	hypothetical protein IscW_ISCW016627 [Ixodes scapularis]	15	9338	11.95	R.ERPFGAGR.E
gi|158297905	AGAP004765-PA [Anopheles gambiae str. PEST]	15	82488	6.3	K.MLKGEYTLK.A
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	15	66947	8.64	K.ILKNLAGKPK.I
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	14	47623	7.81	R.LLVASQPEVLI.-
gi|115712231	PREDICTED: similar to LOC495952 protein, partial [Strongylocentrotus purpuratus]	14	41446	6.24	R.LDGKGQGGSK.G

Appendix Table A18 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|242003880	voltage and ligand gated potassium channel, putative [Pediculus humanus corporis]	14	225218	9.13	K.FLIKFFPEDMK.Y + Oxidation (M)
gi|170580994	U1 zinc finger family protein [Brugia malayi]	14	51370	7.07	K.IDGPIEFR.K
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	14	23318	10.58	K.ENQKPIKPK.T
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	14	225555	5.77	K.DQVGQVNPPK.Y
gi|193650221	PREDICTED: similar to Sodium channel modifier 1 [Acyrthosiphon pisum]	14	24411	8.91	K.TKLELPEDDSDNFK.E

Appendix Table A19 Protein identification data form LCMS analysis. This table presented data of gel number 7 (G7) which found protein bands in tick’s gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|33318285	beta-actin [Tigriopus japonicus]	33	42098	5.3	K.SYELPDGQVITIGNER.F K.LCYVALDFEQEMGTAASSSSLEK.S + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	28	48763	5.94	K.LGGEKPAKPK.E
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	28	60310	6.56	K.TYFKIQPK.L
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	28	125426	9.1	R.VGINRLKPK.L
gi|170586404	F-box domain containing protein [Brugia malayi]	28	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	28	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	28	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	28	150695	6.9	K.SCEYIKPK.E
gi|224305	actin	28	41827	5.3	K.SYELPDGQVITIGNER.F K.LCYVALDFENEMATAASSSSLEK.S + Oxidation (M)
gi|42560382	actin [Stictodora lari]	27	22942	4.92	K.SYELPDGQVITIGNER.F K.LCYVALDFEQEMGTASASSSLEK.S + Oxidation (M)
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	26	78492	8.75	R.SPELLLQPK.T
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	25	406489	5.99	K.EPGQLQQLLR.D
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	25	112787	4.31	K.LETKGLFMSR.L
gi|156400082	predicted protein [Nematostella vectensis]	25	62244	9.78	K.EDKLLDHR.D
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	24	85695	7.94	K.AATPSPKQPK.E

Appendix Table A19 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	24	99637	4.8	K.ALLNGLKAEPR.V
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	23	66947	8.64	K.ILKNLAGKPK.I
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	23	23318	10.58	K.ENQKPIKPK.T
gi|268556740	Hypothetical protein CBG08656 [Caenorhabditis briggsae]	22	46743	5.75	-.DQIKMASEWGYNQEDIIAALK.E + Oxidation (M)
gi|728792	RecName: Full=Actin-3	22	42123	5.29	K.SYELPDGQVITIGNER.F K.LCYVALDFDQEMATAASSSSLEK.S + Oxidation (M)
gi|170579315	hypothetical protein [Brugia malayi]	21	101112	5.54	K.QQEESDSQDNADVDSDDIITSK.L
gi|242003806	conserved hypothetical protein [Pediculus humanus corporis]	21	86129	5.61	R.EKYCPLVTNGEGMELLEELAK.A + Oxidation (M)
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	19	174650	5.27	R.GAPGLSGLKGPK.G
gi|121256	RecName: Full=Globin CTT-IIIA	19	16144	6.11	K.ALLNQLGTSHK.A
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	18	44167	8.9	K.MPGPTVVPIR.E + Oxidation (M)
gi|268561914	Hypothetical protein CBG18668 [Caenorhabditis briggsae]	18	32466	9.51	R.IESAAPLPTEPR.G
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	18	101408	5.79	K.KATEYGSTPK.W
gi|170594123	rad50 family protein [Brugia malayi]	18	155446	6.32	R.ITEGRLPLLIR.Q
gi|170043935	conserved hypothetical protein [Culex quinquefasciatus]	18	28184	6.84	K.IVSTIFAAPPSGTYEEALDCFEK.A
gi|157120630	hypothetical protein AaeL_AAEL009087 [Aedes aegypti]	18	31539	6.01	R.YHMVIHGPK.K

Appendix Table A19 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|157125330	nadph oxidase [Aedes aegypti]	18	56573	9.55	K.SVTVFSCGPK.G
gi|156363128	predicted protein [Nematostella vectensis]	17	37562	6.31	K.FPVRPLIIK.T
gi|115659	RecName: Full=Short-chain collagen C4	17	36445	4.58	K.GDTGAPGPQGPK.G
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	17	194679	8.93	R.RVSEGNAPHSK.N
gi|91088505	PREDICTED: similar to melanin-inhibiting protein [Tribolium castaneum]	17	40857	4.78	K.DLASLPLKPK.A
gi|115751490	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	17	56135	6.87	K.RSCEIASTSVLSPGTQTSFPWK.Q
gi|91080495	PREDICTED: similar to UPF0493 protein KIAA1632 homolog [Tribolium castaneum]	16	270081	6.4	K.ILQLQVGANPAR.G
gi|115679221	PREDICTED: similar to DEAH (Asp-Glu-Ala-His) box polypeptide 38, partial [Strongylocentrotus purpuratus]	16	137807	6.38	K.DIMDQQGMELVSSGTSWDLIR.K
gi|256089895	hypothetical protein [Schistosoma mansoni]	16	8062	6.23	R.SELQIEQFINSTRTELFDLR.N
gi|156540708	PREDICTED: similar to tyrosine recombinase [Nasonia vitripennis]	15	62808	8.95	K.NQTKLLLSLYK.E
gi|113215	RecName: Full=Actin, clone 205	15	42158	5.3	K.SYELPDGQVLTIGNER.F
gi|66516140	PREDICTED: similar to CG10877-PA [Apis mellifera]	15	47702	8.87	K.TNKEWAIVFEGASFPYGPINTIK.Q
gi|115741829	PREDICTED: similar to RAB6 interacting protein 1 [Strongylocentrotus purpuratus]	15	186871	6.67	R.VEVYDNGQWGTVCNDLWDIR.N
gi|66506328	PREDICTED: similar to CG6015-PA [Apis mellifera]	15	66747	6.22	R.ESEVNNMIDSTNVVNSIFAK.N
gi|33520669	cytochrome c oxidase subunit I [Cryptops spinipes]	15	22444	5	-.MALVLGTWSAMLGTAFSLIIR.L + Oxidation (M)

Appendix Table A19 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156545730	PREDICTED: similar to conserved hypothetical protein [Nasonia vitripennis]	14	29746	9.74	K.KVPGYDVK.D
gi|156543280	PREDICTED: hypothetical protein [Nasonia vitripennis]	14	129511	8.27	K.HSDSLLKILR.N
gi|241033935	hypothetical protein IscW_ISCW001706 [Ixodes scapularis]	14	81418	9.14	K.LLKVIVLTEPR.M
gi|256071293	hypothetical protein [Schistosoma mansoni]	14	47124	5.01	K.GFQPPEDYNEMTCFMCIK.K
gi|196013073	hypothetical protein TRIADDRAFT_64279 [Trichoplax adhaerens]	14	42943	6.01	K.VAESFECKPIEGMMSHQLKK.Y + 2 Oxidation (M)

Appendix Table A20 Protein identification data form LCMS analysis. This table presented data of gel number 7 (G7) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	42	42046	8.57	-.MAALTKGVFIVAAK.R
gi|164472575	BA86-like protein [Rhipicephalus annulatus]	31	71481	5.72	K.VLCECPWNQHLVGDK.C
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	31	34737	8.06	K.IINLAQGKPK.G
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	30	58116	9.25	R.YHQKPLAPK.V
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	26	66947	8.64	K.ILKNLAGKPK.I
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	25	112787	4.31	K.LETKGLFMSR.L
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	23	174650	5.27	R.GAPGLSGLKGPK.G
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	23	20283	7.79	R.GNGGGGGGGGGSR.G
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	23	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	22	194679	8.93	R.RVSEGNAPHSK.N
gi|268562225	Hypothetical protein CBG05575 [Caenorhabditis briggsae]	22	18215	4.75	K.VVRSPNKPGK.G
gi|146197849	hnRNP K protein [Dugesia japonica]	21	38051	8.04	K.VVGAIIGPGGSR.I
gi|170580994	U1 zinc finger family protein [Brugia malayi]	21	51370	7.07	K.IDGPIEFR.K
gi|254933957	carbamoylphosphate synthetase [Euclemensia bassettella]	21	109474	6.04	K.WLESYTHSSITFEILKGAK.Q
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	20	121129	8.33	K.GGGGGGGGGGGGDK.K

Appendix Table A20 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|189236207	PREDICTED: similar to GA19476-PA [Tribolium castaneum]	20	75172	5.46	K.IDELSGDMMNSGAITFSAKPGR.G + Oxidation (M)
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	20	9092	9.51	-.MECLRSVLTIAGK.A
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	19	56983	9.9	-.TLTHRVR.L
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	19	65748	9.67	R.TITHSPVK.S
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	19	165556	6.1	K.MVLPRLQIIR.G
gi|157117164	coracle protein, putative [Aedes aegypti]	19	180952	6.64	K.DNGQITIVGVTDPKTHK.V
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	19	16163	9.54	-.MAALLLQLNSCSR.K
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	18	165126	9.32	R.ILLDQRVGAPR.Y
gi|120660499	cytochrome oxidase subunit II [Crematogaster sp. D SPQ-2003]	18	21886	6.17	R.ILTTSLDVIHSWTVPSLGIKXXSTPGR.X
gi|268552739	Hypothetical protein CBG17702 [Caenorhabditis briggsae]	18	55277	9.63	R.DSAITGEQINEDK.I
gi|3608420	cyclin A [Dreissena polymorpha]	18	47955	5.76	R.VQPSRAAKPK.V
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	18	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	18	101408	5.79	K.KATEYGSTPK.W
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	18	67527	9.56	K.AQINVALKIIR.N
gi|157120630	hypothetical protein AaeL_AAEL009087 [Aedes aegypti]	17	31539	6.01	R.YHMVIHGPK.K

Appendix Table A20 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156544936	PREDICTED: hypothetical protein [Nasonia vitripennis]	17	18225	7.88	R.MPTGPDGTKGFNVR.R
gi|256075113	brefeldin A-inhibited guanine nucleotide-exchange protein [Schistosoma mansoni]	17	230732	5.6	R.LSKSHIGHFLGENEPYNLR.V
gi|72099627	PREDICTED: similar to MGC83346 protein [Strongylocentrotus purpuratus]	17	66356	6.37	K.WLGCQAMDNQIVIYSAINR.F + Oxidation (M)
gi|72089047	PREDICTED: similar to Ehf protein [Strongylocentrotus purpuratus]	17	57403	5.96	K.NNPQMTYEK.L
gi|220961601	cytochrome oxidase subunit I [Nasonia longicornis]	17	28370	7.1	M.GSINFATTVLNMK.I + Oxidation (M)
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	17	48812	8.57	K.SQKYGHVK.S
gi|156364690	predicted protein [Nematostella vectensis]	16	61422	6.17	K.IEQDLWNSAFKSQIDFLR.Q
gi|157125153	hypothetical protein AaeL_AAEL010109 [Aedes aegypti]	16	20735	6.54	K.TISTVMNT.- + Oxidation (M)
gi|268570016	Hypothetical protein CBG24648 [Caenorhabditis briggsae]	16	39526	6.38	K.DDVVVGEMFEMVEAEEVQKHYEGSK.L + Oxidation (M)
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	16	126171	7.14	R.IITLLRQIIR.T
gi|170574856	Protein kinase domain containing protein [Brugia malayi]	16	26666	9.93	R.DLDHNDQLLR.I
gi|268559262	C. briggsae CBR-NASP-2 protein [Caenorhabditis briggsae]	16	60962	4.21	-.MSALADTTNTITQEKETVEK.V
gi|268530264	Hypothetical protein CBG00680 [Caenorhabditis briggsae]	16	68201	8.68	K.IKSEMSPMTVPVSPMADENR.L + 3 Oxidation (M)
gi|170047980	copper-transporting ATPase 1 [Culex quinquefasciatus]	16	135857	5.48	K.LFGATSKVSTEDR.L
gi|242003208	histone H3, putative [Pediculus humanus corporis]	16	17648	11.34	-.MRPMKSLTGNDVANEK.R + 2 Oxidation (M)

Appendix Table A20 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	16	110364	5.32	K.MNPFNVPK.T
gi|91081233	PREDICTED: similar to hormone-sensitive lipase [Tribolium castaneum]	16	84776	5.09	K.YKSKPPAR.G
gi|156406821	predicted protein [Nematostella vectensis]	16	49756	4.94	K.HEEEEAEHLWEEFLSPAK.L
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	16	115140	8.72	K.MGPPMQGR.G + Oxidation (M)
gi|170047092	serrate protein [Culex quinquefasciatus]	16	419035	5.51	R.IRLGCTAR.Y

Appendix Table A21 Protein identification data form LCMS analysis. This table presented data of gel number 7 (G7) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|241694276	conserved hypothetical protein [Ixodes scapularis]	35	34468	8.14	R.GGYLVKDLK.S
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	35	60310	6.56	K.TYFKIQPK.L
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	35	125426	9.1	R.VGINRLKPK.L
gi|170586404	F-box domain containing protein [Brugia malayi]	35	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	35	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	35	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	35	150695	6.9	K.SCEYIKPK.E
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	33	78492	8.75	R.SPELLLQPK.T
gi|91081321	PREDICTED: similar to CG5198 CG5198-PA [Tribolium castaneum]	31	36861	4.72	K.MLNYMKPK.E
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	31	85695	7.94	K.AATPSPKQPK.E
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	31	40519	6.4	K.EGGPPKSKPK.K
gi|256078034	initiation factor 5a [Schistosoma mansoni]	31	17855	5.41	K.DYQLVDIK.D
gi|242023447	conserved hypothetical protein [Pediculus humanus corporis]	28	48829	8.68	K.DFIENSLR.K
gi|268572719	Hypothetical protein CBG13254 [Caenorhabditis briggsae]	28	55921	6.59	K.NLMEITQK.F + Oxidation (M)
gi|91090029	PREDICTED: similar to glycerol-3-phosphate acyltransferase [Tribolium castaneum]	28	98975	6.87	R.NFLKDDLK.E

Appendix Table A21 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|158301484	AGAP001899-PA [Anopheles gambiae str. PEST]	28	266844	5.81	R.NMLDKDIK.S + Oxidation (M)
gi|158294544	AGAP005651-PA [Anopheles gambiae str. PEST]	28	47687	5.78	R.NIFEDLNK.-
gi|242021826	Angiotensin-converting enzyme precursor, putative [Pediculus humanus corporis]	28	81610	9.34	K.NYILLSNR.A
gi|196015769	hypothetical protein TRIADDRAFT_33038 [Trichoplax adhaerens]	27	479542	5.78	K.LNFENDLK.I
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	27	34737	8.06	K.IINLAQGKPK.G
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	27	112787	4.31	K.LETKGLFMSR.L
gi|193652752	PREDICTED: similar to exosome complex exonuclease RRP44 [Acyrthosiphon pisum]	26	94487	6.53	M.DFLENLSR.Q
gi|118781575	AGAP010405-PA [Anopheles gambiae str. PEST]	24	17503	9.32	K.TAQSMMRPK.S + 2 Oxidation (M)
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	23	24881	9.76	K.RADPGIFAWEIR.D
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	23	99637	4.8	K.ALLNGLKAEPR.V
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	22	66947	8.64	K.ILKNLAGKPK.I
gi|20799320	fasciclin-like protein [Aplysia californica]	22	87461	7	R.INVIDDVMVPPK.G + Oxidation (M)
gi|157713480	nicotinic acetylcholine receptor beta subunit [Haemonchus contortus]	21	55417	6.19	R.VDEQKHLQK.L
gi|170029771	lupus la ribonucleoprotein [Culex quinquefasciatus]	21	185947	9.51	K.WVPLPIDLPKPR.A
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	21	174650	5.27	R.GAPGLSGLKGPK.G

Appendix Table A21 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156366213	predicted protein [Nematostella vectensis]	21	56054	8.98	K.GAAGSRPPSPER.D
gi|256073417	hypothetical protein [Schistosoma mansoni]	20	51173	9.54	R.LVQRLDSMISLR.R
gi|115659	RecName: Full=Short-chain collagen C4	20	36445	4.58	K.GDTGAPGPQGPK.G
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	19	6635	9.81	K.TITDNKEMK.T
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	19	34876	7.64	R.RGTDNAAVFK.F
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	19	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|193681067	PREDICTED: similar to conserved hypothetical protein [Acyrthosiphon pisum]	19	43752	8.39	K.SVDKSEYVR.Y
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	19	58116	9.25	R.YHQKPLAPK.V
gi|156546758	PREDICTED: similar to ATP-binding cassette sub-family A member 3, putative [Nasonia vitripennis]	18	187844	8.66	R.MPNEFYDVACK.S
gi|268577861	Hypothetical protein CBG02177 [Caenorhabditis briggsae]	18	76713	6.36	R.LPNPLLSVAR.E
gi|115768164	PREDICTED: similar to megalin [Strongylocentrotus purpuratus]	18	189249	4.36	K.MIAKTIQMK.M + Oxidation (M)
gi|157106621	DNA polymerase eta [Aedes aegypti]	18	95165	7.49	R.KRPTSGDK.S
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	18	101408	5.79	K.KATEYGSTPK.W
gi|170035015	roadkill E [Culex quinquefasciatus]	18	43094	9.11	R.RENPDLVAEIMK.K + Oxidation (M)
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	18	406489	5.99	K.EPGQLQQLLR.D

Appendix Table A21 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|256085548	afadin (af-6 protein) [Schistosoma mansoni]	18	235964	8.69	K.FHPDLRMLTAGR.Y + Oxidation (M)
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	18	56983	9.9	-.TLTHRVR.L
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	18	65748	9.67	R.TITHSPVK.S
gi|157104804	hypothetical protein AaeL_AAEL004171 [Aedes aegypti]	18	75971	6.24	K.QTFKRPVPK.F
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	18	194679	8.93	R.RVSEGNAPHSK.N

Appendix Table A22 Protein identification data form LCMS analysis. This table presented data of gel number 8 (G8) which found protein bands in tick’s gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|91077092	PREDICTED: similar to predicted protein [Tribolium castaneum]	48	93691	9.05	R.KVQANIAR.K R.LLPTNELPER.L
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	43	406489	5.99	K.EPGQLQQLLR.D
gi|156408383	predicted protein [Nematostella vectensis]	43	56096	8.67	R.HSQVNSKPER.G
gi|195998638	hypothetical protein TRIADDRAFT_21077 [Trichoplax adhaerens]	33	36004	9.91	R.DYNNKVIK.K
gi|256087255	smg-7 (suppressor with morphological effect on genitalia protein 7) [Schistosoma mansoni]	33	170574	6.22	K.NLEFDINK.S
gi|242006382	conserved hypothetical protein [Pediculus humanus corporis]	32	57741	5.9	K.TNIPKVKPER.S
gi|221109683	PREDICTED: similar to Aof2 protein [Hydra magnipapillata]	32	82082	9.34	K.NYDKLVLK.I
gi|256078034	initiation factor 5a [Schistosoma mansoni]	31	17855	5.41	K.DYQLVDIK.D
gi|170039873	conserved hypothetical protein [Culex quinquefasciatus]	30	42801	10.02	R.AMRFFAGPER.S
gi|170592699	ABC transporter family protein [Brugia malayi]	29	253101	8.2	R.DNYLIDLK.Y
gi|196005577	hypothetical protein TRIADDRAFT_56895 [Trichoplax adhaerens]	29	72241	9.49	K.NDVLSSVMK.A + Oxidation (M)
gi|189235485	PREDICTED: similar to dynein heavy chain [Tribolium castaneum]	28	543239	5.76	R.KVVDVLVK.V
gi|225709224	Probable mitochondrial 28S ribosomal protein S6 [Caligus rogercresseyi]	27	17331	9.62	R.RFPQTTEFR.G
gi|66507736	PREDICTED: similar to Elongin B CG4204-PA [Apis mellifera]	27	13138	4.85	K.DNVLMSDSK.F
gi|170051303	aminopeptidase N [Culex quinquefasciatus]	27	104069	5.9	R.AASVLNMFR.Q

Appendix Table A22 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170586382	DEAH [Brugia malayi]	27	77562	9.2	K.KTVLNMFR.D
gi|241694276	conserved hypothetical protein [Ixodes scapularis]	27	34468	8.14	R.GGYLVKDLK.S
gi|158294544	AGAP005651-PA [Anopheles gambiae str. PEST]	27	47687	5.78	R.NIFEDLNK.-
gi|242021826	Angiotensin-converting enzyme precursor, putative [Pediculus humanus corporis]	26	81610	9.34	K.NYILLSNR.A
gi|115942961	PREDICTED: similar to KIAA1636 protein [Strongylocentrotus purpuratus]	26	247346	9.11	R.NIFFPNIK.V
gi|170574648	Sly1 protein homolog [Brugia malayi]	25	71079	5.68	K.LDVLFEAEEK.I
gi|240270422	serpin 10 plasmodium-related inhibitory serine protease inhibitor [Anopheles gambiae]	25	24471	5.25	K.QTVIDNYR.R
gi|193652752	PREDICTED: similar to exosome complex exonuclease RRP44 [Acyrthosiphon pisum]	25	94487	6.53	M.DFLENLSR.Q
gi|241186228	sdk-P1, putative [Ixodes scapularis]	25	191031	5.99	R.EGVPQAAPSGLR.A
gi|193639991	PREDICTED: similar to AGAP012271-PA [Acyrthosiphon pisum]	25	39589	9.25	K.TGAIVEHPGK.D
gi|78190779	chaperonin complex component TCP-1 beta subunit [Aphrocallistes vastus]	24	29655	6.52	R.QAEVLIAR.K
gi|156349294	predicted protein [Nematostella vectensis]	23	43681	11.48	R.VMPAPVK.Q + Oxidation (M)
gi|115610702	PREDICTED: similar to DEP domain containing 5 [Strongylocentrotus purpuratus]	23	182684	6.83	R.DNVKVDYAGR.F
gi|270008026	hypothetical protein TcasGA2_TC014778 [Tribolium castaneum]	23	11578	11.55	K.GLSLGLIAR.Y
gi|110764993	PREDICTED: similar to atypical protein kinase C CG10261-PA isoform 1 [Apis mellifera]	23	68918	5.7	K.AASVLKGFLCK.D

Appendix Table A22 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|91077070	PREDICTED: similar to ancient conserved domain protein 2 (cyclin m2) [Tribolium castaneum]	23	105903	5.7	K.KTVADVMTK.I + Oxidation (M)
gi|226487542	hypotheticial protein [Schistosoma japonicum]	23	58387	9.34	R.INLSSLPR.S
gi|156543116	PREDICTED: similar to ENSANGP00000011728 [Nasonia vitripennis]	23	109068	6.37	K.RAISSPIR.S
gi|170058242	conserved hypothetical protein [Culex quinquefasciatus]	23	100536	6.83	R.QVLETGPR.G
gi|115746600	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	23	141703	9.95	R.KTPPVAASIPGR.R
gi|5107897	cytochrome c oxidase subunit I [Cafferia caffer]	22	21320	4.76	-.LIGLALSLLIR.A
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	22	20283	7.79	R.GNGGGGGGGGGSR.G
gi|268563166	C. briggsae CBR-WWP-1 protein [Caenorhabditis briggsae]	22	93668	6.92	K.VKLTSVPR.N
gi|268566075	Hypothetical protein CBG12340 [Caenorhabditis briggsae]	22	38692	9.38	R.IDVIEGKSFK.T
gi|157123807	golgi-specific brefeldin a-resistance factor [Aedes aegypti]	22	214572	5.46	K.DIVLLLFMR.L + Oxidation (M)
gi|242023511	conserved hypothetical protein [Pediculus humanus corporis]	22	52080	9.65	R.NDMEKNIK.C + Oxidation (M)
gi|241155753	excitatory amino acid transporter, putative [Ixodes scapularis]	21	102695	6.09	K.DIVIRHVR.D
gi|170594801	U88 [Brugia malayi]	21	8487	7.42	M.NLTLVIAR.M
gi|241561644	synembryn, putative [Ixodes scapularis]	21	14334	4.73	K.LARGGVVQPAR.V
gi|156342124	hypothetical protein NEMVEDRAFT_v1g222611 [Nematostella vectensis]	21	46767	8.81	K.SKQAPAR.V

Appendix Table A22 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|91077070	PREDICTED: similar to ancient conserved domain protein 2 (cyclin m2) [Tribolium castaneum]	23	105903	5.7	K.KTVADVMTK.I + Oxidation (M)
gi|226487542	hypotheticial protein [Schistosoma japonicum]	23	58387	9.34	R.INLSSLPR.S
gi|156543116	PREDICTED: similar to ENSANGP00000011728 [Nasonia vitripennis]	23	109068	6.37	K.RAISSPIR.S
gi|170058242	conserved hypothetical protein [Culex quinquefasciatus]	23	100536	6.83	R.QVLETGPR.G
gi|115746600	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	23	141703	9.95	R.KTPPVAASIPGR.R

Appendix Table A23 Protein identification data form LCMS analysis. This table presented data of gel number 8 (G8) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|34500282	Bm95 protein [Rhipicephalus microplus]	42	67154	6.44	K.ANCQCPPDTKPGEIGCIER.T
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	31	40519	6.4	K.EGGPPKSKPK.K
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	31	85695	7.94	K.AATPSPKQPK.E
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	25	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|158291121	AGAP002345-PA [Anopheles gambiae str. PEST]	23	87465	5.38	R.QCGAIFPHHENMSLLQQQK.L + Oxidation (M)
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	23	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|15298096	green fluorescent protein [Montastraea cavernosa]	23	26002	6.09	K.LRMQGVVNGHK.F
gi|37703935	elongation factor-2 [Eurytemora affinis]	22	79396	5.78	K.LTTXXGKXVK.D
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	22	112787	4.31	K.LETKGLFMSR.L
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|115741974	PREDICTED: similar to CG7628-PA [Strongylocentrotus purpuratus]	21	58368	5.72	K.ENPLEPGLR.V
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	21	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F

Appendix Table A23 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|34500282	Bm95 protein [Rhipicephalus microplus]	42	67154	6.44	K.ANCQCPPDTKPGEIGCIER.T
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	31	40519	6.4	K.EGGPPKSKPK.K
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	31	85695	7.94	K.AATPSPKQPK.E
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	25	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|158291121	AGAP002345-PA [Anopheles gambiae str. PEST]	23	87465	5.38	R.QCGAIFPHHENMSLLQQQK.L + Oxidation (M)
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	23	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|15298096	green fluorescent protein [Montastraea cavernosa]	23	26002	6.09	K.LRMQGVVNGHK.F
gi|37703935	elongation factor-2 [Eurytemora affinis]	22	79396	5.78	K.LTTXXGKXVK.D
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	22	112787	4.31	K.LETKGLFMSR.L
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|115741974	PREDICTED: similar to CG7628-PA [Strongylocentrotus purpuratus]	21	58368	5.72	K.ENPLEPGLR.V
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	21	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F

Appendix Table A23 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	17	44167	8.9	K.MPGPTVVPIR.E + Oxidation (M)
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	17	6635	9.81	K.TITDNKEMK.T
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	17	34876	7.64	R.RGTDNAAVFK.F
gi|31239659	cytochrome P450 (AGAP012295-PA) [Anopheles gambiae str. PEST]	17	62060	7.61	K.IMGYQVFPK.L
gi|157125153	hypothetical protein AaeL_AAEL010109 [Aedes aegypti]	16	20735	6.54	K.TISTVMNT.- + Oxidation (M)
gi|196012718	hypothetical protein TRIADDRAFT_60295 [Trichoplax adhaerens]	16	78546	6.06	R.LLDTTTTDAK.E
gi|196002387	predicted protein [Trichoplax adhaerens]	16	122670	9.24	K.NLTRPAGVGIIK.G
gi|156408321	predicted protein [Nematostella vectensis]	16	109267	8.68	K.TAVTNTGASNTR.I
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	16	47623	7.81	R.LLVASQPEVLI.-
gi|157120630	hypothetical protein AaeL_AAEL009087 [Aedes aegypti]	16	31539	6.01	R.YHMVIHGPK.K
gi|115659	RecName: Full=Short-chain collagen C4	16	36445	4.58	K.GDTGAPGPQGPK.G
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	16	138300	5.84	K.LMVKYDDIK.I
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	16	406489	5.99	K.EPGQLQQLLR.D
gi|242010006	hypothetical protein Phum_PHUM219920 [Pediculus humanus corporis]	16	27221	9.21	K.ILPCSLTMK.S + Oxidation (M)
gi|98960841	fibroblast growth factor receptor [Spodoptera frugiperda]	16	97166	5.85	K.AECVGILKPGMQSVVAVKMLK.E

Appendix Table A23 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|196014839	hypothetical protein TRIADDRAFT_32175 [Trichoplax adhaerens]	16	55916	6.29	K.TMDTTGAHVIQLETAIGAAIK.C
gi|193702219	PREDICTED: similar to DNA-repair protein XRCC1 (X-ray repair cross-complementing protein 1) [Acyrthosiphon pisum]	16	61790	7.24	K.NDTITMAAVK.N + Oxidation (M)
gi|91076832	PREDICTED: similar to GA20540-PA [Tribolium castaneum]	16	145934	7.49	R.VQSEFDQVK.L
gi|193688066	PREDICTED: similar to stall CG3622-PC [Acyrthosiphon pisum]	16	77642	5.7	R.TRTCNNPKPINTDEGCEGPR.H
gi|268576404	C. briggsae CBR-OSM-10 protein [Caenorhabditis briggsae]	15	44738	8.58	K.SSESNGVSNMTSHAK.N + Oxidation (M)

Appendix Table A24 Protein identification data form LCMS analysis. This table presented data of gel number 8 (G8) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	29	22842	5.43	M.ASLTIPKAPK.K
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	28	112787	4.31	K.LETKGLFMSR.L
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	28	85695	7394	K.AATPSPKQPK.E
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	28	40519	6.4	K.EGGPPKSKPK.K
gi|268564031	Hypothetical protein CBG03598 [Caenorhabditis briggsae]	26	31520	5.95	K.QTVISDFKK.N
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	25	75672	9.35	K.TLTFEEIRK.K
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	25	34737	8.06	K.IINLAQGKPK.G
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	24	20283	7.79	R.GNGGGGGGGGGSR.G
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	24	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|91088267	PREDICTED: similar to Tumor suppressor candidate 4 (NPR2-like) (Gene 21 protein) (G21 protein) [Tribolium castaneum]	24	44347	6.13	K.AATKLAPILK.Q
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	23	66947	8.64	K.ILKNLAGKPK.I
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	22	165556	6.1	K.MVLPRLQIIR.G
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	22	67527	9.56	K.AQINVALKIIR.N
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	22	225555	5.77	K.DQVGQVNPPK.Y
gi|156363128	predicted protein [Nematostella vectensis]	22	37562	6.31	K.FPVRPLIIK.T

Appendix Table A24 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|170586404	F-box domain containing protein [Brugia malayi]	21	53036	9.66	R.QNALLIQPK.A
gi|196006884	hypothetical protein TRIADDRAFT_7238 [Trichoplax adhaerens]	21	15711	9.38	K.TAAKGSPFFR.K
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	21	22059	6.92	M.ENKLDSYR.K
gi|156372629	predicted protein [Nematostella vectensis]	21	99962	7.9	R.LDVNANTYR.N
gi|48122204	PREDICTED: similar to Mediator of RNA polymerase II transcription subunit 31 (Mediator complex subunit SOH1) [Apis mellifera]	21	18940	8.94	R.EVVNSQCTK.F
gi|158301517	AGAP001877-PA [Anopheles gambiae str. PEST]	20	90145	6.37	K.LTIKPMPPK.E
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	20	194679	8.93	R.RVSEGNAPHSK.N
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	20	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	20	6635	9.81	K.TITDNKEMK.T
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	20	34876	7.64	R.RGTDNAAVFK.F
gi|170059237	conserved hypothetical protein [Culex quinquefasciatus]	20	117287	5.27	K.TLKTIGGYVK.N
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	19	23318	10.58	K.ENQKPIKPK.T
gi|268578537	Hypothetical protein CBG13997 [Caenorhabditis briggsae]	19	25220	9.59	K.LLKIEQLLL.-
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	19	129521	6.67	R.LTTSMILER.L + Oxidation (M)

Appendix Table A24 (Continued)

Accession number	**Protein Hits**	**Protein score**	**Mw**	**PI**	**Peptide sequence**
gi|221122039	PREDICTED: similar to TNF-receptor-associated factor 1 [Hydra magnipapillata]	19	44059	6.6	R.EPVLTLCGHR.L
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	19	165126	9.32	R.ILLDQRVGAPR.Y
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	19	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|187234601	carbamoylphosphate synthetase/aspartate transcarbamylase/dihydroorotase [Sphinx dollii]	19	109763	5.98	K.FVPDIHERPK.K
gi|66530381	PREDICTED: similar to dynein, axonemal, heavy polypeptide 3 [Apis mellifera]	18	435094	5.4	K.ILKVGTLIPK.I
gi|254934465	period [Acraga philetera]	18	36907	8.57	K.LDVIIAKHR.V
gi|18307516	putative venom metalloprotease [Pimpla hypochondriaca]	18	61759	6.79	R.CTHSRPVGVPK.G
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|114052452	NADH dehydrogenase isoform 1 [Bombyx mori]	18	17284	5.93	K.DIVQYIADK.E
gi|196012718	hypothetical protein TRIADDRAFT_60295 [Trichoplax adhaerens]	18	78546	6.06	R.LLDTTTTDAK.E
gi|262304099	phosphogluconate dehydrogenase [Colossendeis sp. Col]	18	28326	6.25	K.VLAGPENTK.F
gi|158287207	AGAP011349-PA [Anopheles gambiae str. PEST]	17	65162	9.04	R.SERLPQQPK.V
gi|170588453	oxidoreductase, short chain dehydrogenase/reductase family protein [Brugia malayi]	17	44081	6.85	K.GIGKAIAVALIR.R
gi|242011894	predicted protein [Pediculus humanus corporis]	17	210112	7.99	R.NEQAGAAAHIISENIK.M
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	16	159499	5.87	R.DVTETLSTDV.-

Appendix Table A24 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|256088135	phosphatidylinositol-45-bisphosphate 3-kinase catalytic subunit alpha PI3K [Schistosoma mansoni]	16	230421	6.44	R.LDVLIKHAR.C
gi|115681464	PREDICTED: similar to titin isoform N2-A [Strongylocentrotus purpuratus]	16	1214953	5.59	K.EVVPSDKYK.M
gi|115623592	PREDICTED: similar to ankyrin 2,3/unc44 [Strongylocentrotus purpuratus]	16	146295	5.37	R.DIVKYLVSK.G
gi|256082211	ribc1 [Schistosoma mansoni]	16	45664	7.56	R.DLVYAADAAR.N
gi|66522082	PREDICTED: similar to GMP synthase [glutamine-hydrolyzing] (Glutamine amidotransferase) (GMP synthetase) [Apis mellifera]	16	79444	7.06	R.VEGATSESER.Q

Appendix Table A25 Protein identification data form LCMS analysis. This table presented data of gel number 9 (G9) which found protein bands in tick's gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|60691514	SJCHGC05193 protein [Schistosoma japonicum]	31	9688	9.7	R.DKQPNGPLR.S
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	30	22059	6.92	M.ENKLDSYR.K
gi|156540439	PREDICTED: hypothetical protein [Nasonia vitripennis]	22	161841	6.18	R.EGGEPIPVAR.L
gi|242004760	predicted protein [Pediculus humanus corporis]	22	97578	9.35	K.VEVIGTPQHR.A
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	22	194679	8.93	R.RVSEGNAPHSK.N
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	22	23318	10.58	K.ENQKPIKPK.T
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	22	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|221120648	PREDICTED: similar to viral A-type inclusion protein, partial [Hydra magnipapillata]	21	147762	7.31	K.WGKDLYSR.N
gi|66514180	PREDICTED: similar to 5-3 exoribonuclease 1 isoform 1 [Apis mellifera]	21	189805	8.54	K.NENHLPIEAAMSPK.W
gi|157115340	hypothetical protein AaeL_AAEL007155 [Aedes aegypti]	21	30340	6.64	K.IDKLLAKPK.H
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|170045498	BTB/POZ domain-containing protein 2 [Culex quinquefasciatus]	21	44305	7.97	R.RSQANVPPR.D
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	20	126171	7.14	R.IITLLRQIIR.T
gi|256076522	gamma-tubulin complex component 4 (gcp-4) [Schistosoma mansoni]	20	85920	5.68	-.MLHELLLALHGISGGIFVQSDK.T + Oxidation (M)

Appendix Table A25 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170046628	U3 small nucleolar ribonucleoprotein protein MPP10 [Culex quinquefasciatus]	20	75364	4.67	K.LLEKGIKPK.G
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	19	159499	5.87	R.DVTETLSTDV.-
gi|268566075	Hypothetical protein CBG12340 [Caenorhabditis briggsae]	19	38692	9.38	R.IDVIEGKSFK.T
gi|37703935	elongation factor-2 [Eurytemora affinis]	19	79396	5.78	K.LTTXXGKXVK.D
gi|242010291	ubiquitin specific protease, putative [Pediculus humanus corporis]	19	409272	5.13	K.NNGLAKDSK.T
gi|170029975	golgi-specific brefeldin a-resistance factor [Culex quinquefasciatus]	19	210069	5.38	K.DIVLLLFMR.L + Oxidation (M) / K.VTLLREQNLVPK.T
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	18	112787	4.31	K.LETKGLFMSR.L
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	18	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	18	48812	8.57	K.SQKYGHVK.S
gi|157106434	hypothetical protein AaeL_AAEL004539 [Aedes aegypti]	18	6257	12.01	R.LDTAMAAHVKPILR.L + Oxidation (M)
gi|76154564	SJCHGC05035 protein [Schistosoma japonicum]	17	25097	9.24	R.DNVQFVQMR.R
gi|115610702	PREDICTED: similar to DEP domain containing 5 [Strongylocentrotus purpuratus]	17	182684	6.83	R.DNVKVDYAGR.F
gi|170037179	NGD5 protein [Culex quinquefasciatus]	17	49725	4.82	K.LDVQYEQLK.I
gi|158284931	AGAP002202-PA [Anopheles gambiae str. PEST]	17	26868	6.79	R.LTTDIPYEK.M
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	16	34737	8.06	K.IINLAQGKPK.G

Appendix Table A25 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|256070183	hypothetical protein [Schistosoma mansoni]	16	45798	7.19	K.KPLTRNKPVK.T
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	16	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	16	138300	5.84	K.LMVKYDDIK.I
gi|170590480	60S ribosomal protein L44 [Brugia malayi]	16	67827	9.66	R.KVSDNIMEK.L + Oxidation (M) K.VQDNIMEKLAPR.M + Oxidation (M)
gi|156363128	predicted protein [Nematostella vectensis]	16	37562	6.31	K.FPVRPLIIK.T
gi|170576447	protein F10G7.2 [Brugia malayi]	16	103027	8.19	R.DVEIILEGTSNQNFVGSVIHPK.G
gi|221122797	PREDICTED: similar to Ift46 protein [Hydra magnipapillata]	16	60790	5.47	R.NNDGFLYNPK.D
gi|56755235	SJCHGC05947 protein [Schistosoma japonicum]	16	22965	6.23	-.MWFSGTQPK.T
gi|4218957	cytoplasmic polyadenylation element-binding protein [Spisula solidissima]	15	74011	8.13	R.NTKGHTNLALL.-
gi|268557342	Hypothetical protein CBG23371 [Caenorhabditis briggsae]	15	55812	5.72	K.QRDELSDGYR.N
gi|196004921	hypothetical protein TRIADDRAFT_56227 [Trichoplax adhaerens]	15	15043	7.07	K.LLVEHGGDVNK.G
gi|3023865	RecName: Full=Guanine nucleotide-binding protein G(q) subunit alpha; AltName: Full=Guanine nucleotide-binding protein alpha-q	15	41553	5.18	R.INCEIEK.E
gi|29840923	similar to NM_031954 MSTP028 protein in Homo sapiens [Schistosoma japonicum]	15	39450	6.72	R.NIECLEK.Y
gi|156328522	hypothetical protein NEMVEDRAFT_v1g224666 [Nematostella vectensis]	15	51557	7.95	K.ARECLEK.L
gi|157106155	myosin [Aedes aegypti]	15	180581	8.8	K.NLSSDITR.S

Appendix Table A25 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|242012377	conserved hypothetical protein [Pediculus humanus corporis]	15	45783	6.28	K.NLCELEK.A
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	15	67527	9.56	K.AQINVALKIIR.N
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	15	66947	8.64	K.ILKNLAGKPK.I
gi|115647211	PREDICTED: similar to MGC80611 protein [Strongylocentrotus purpuratus]	15	23945	5.48	K.ELTAGLSQYNK.S
gi|156546770	PREDICTED: similar to rap55 [Nasonia vitripennis]	15	61343	9.32	R.NQQPGGAGVKPK.N

Appendix Table A26 Protein identification data form LCMS analysis. This table presented data of gel number 9 (G9) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|256078034	initiation factor 5a [Schistosoma mansoni]	30	17855	5.41	K.DYQLVDIK.D
gi|268578419	C. briggsae CBR-NUCB-1 protein [Caenorhabditis briggsae]	30	52775	4.97	K.NMNEEDIK.A
gi|189233623	PREDICTED: similar to fibroblast growth factor receptor [Tribolium castaneum]	29	109725	6.63	K.MIDIETVR.A + Oxidation (M)
gi|157134125	hypothetical protein AaeL_AAEL003092 [Aedes aegypti]	28	174099	5.35	R.FDLIEDNK.V
gi|91090029	PREDICTED: similar to glycerol-3-phosphate acyltransferase [Tribolium castaneum]	27	98975	6.87	R.NFLKDDLK.E
gi|158301484	AGAP001899-PA [Anopheles gambiae str. PEST]	27	266844	5.81	R.NMLDKDIK.S + Oxidation (M)
gi|241694276	conserved hypothetical protein [Ixodes scapularis]	27	34468	8.14	R.GGYLVKDLK.S
gi|242023447	conserved hypothetical protein [Pediculus humanus corporis]	27	48829	8.68	K.DFIENSLR.K
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	27	22059	6.92	M.ENKLDSYR.K
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	26	34737	8.06	K.IINLAQGKPK.G
gi|242010126	Nuclear RNA export factor, putative [Pediculus humanus corporis]	26	71855	9.43	R.IVYLEDNK.I
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	26	40519	6.4	K.EGGPPKSKPK.K
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	25	75672	9.35	K.TLTFEEIRK.K
gi|170596587	3'-5' exonuclease family protein [Brugia malayi]	25	81434	6.94	R.AGTVESFAAKR.A
gi|193652752	PREDICTED: similar to exosome complex exonuclease RRP44 [Acyrthosiphon pisum]	25	94487	6.53	M.DFLENLSR.Q

Appendix Table A26 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|115659	RecName: Full=Short-chain collagen C4	24	36445	4.58	K.GDTGAPGPQGPK.G
gi|291235630	PREDICTED: jmjC domain-containing protein 4-like [Saccoglossus kowalevskii]	24	49848	5.12	R.EDQIDDYK.F
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	24	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	22	194679	8.93	R.RVSEGNAPHSK.N
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	21	38467	9.67	K.IKSNPIQPK.F
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	20	174650	5.27	R.GAPGLSGLKGPK.G
gi|110750932	PREDICTED: similar to CG11526-PA, isoform A [Apis mellifera]	20	95261	6.76	-.MRDVAMDANGNGK.R + 2 Oxidation (M)
gi|241557575	conserved hypothetical protein [Ixodes scapularis]	20	30620	9.4	R.HFHESAVGPR.Q
gi|291242540	PREDICTED: regulatory factor X4-like, partial [Saccoglossus kowalevskii]	20	54926	6.73	R.QTVAYSPRSK.L
gi|37703935	elongation factor-2 [Eurytemora affinis]	19	79396	5.78	K.LTTXXGKXVK.D
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	19	159499	5.87	R.DVTETLSTDV.-
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	19	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)

Appendix Table A26 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|156365595	predicted protein [Nematostella vectensis]	19	68246	6.45	R.EDPALGSEHK.T
gi|110762287	PREDICTED: hypothetical protein [Apis mellifera]	19	15790	5.34	R.RSTIFMPNTCK.C
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	19	112787	4.31	K.LETKGLFMSR.L
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	18	126171	7.14	R.IITLLRQIIR.T
gi|15298096	green fluorescent protein [Montastraea cavernosa]	18	26002	6.09	K.LRMQGVVNGHK.F
gi|221114618	PREDICTED: similar to polo-like kinase 4 [Hydra magnipapillata]	18	95333	8.31	R.VVMADYSVPK.Y + Oxidation (M)
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	18	58116	9.25	R.YHQKPLAPK.V
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	17	58245	8.67	K.VRYYQTPVR.D
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	17	24881	9.76	K.RADPGIFAWEIR.D
gi|221126444	PREDICTED: similar to predicted protein [Hydra magnipapillata]	17	78989	7.85	K.DNITTKDSDK.F
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	17	101408	5.79	K.KATEYGSTPK.W
gi|193624948	PREDICTED: similar to gag-like protein [Acyrthosiphon pisum]	17	48472	8.8	R.STLVKEEASFK.R
gi|157123807	golgi-specific brefeldin a-resistance factor [Aedes aegypti]	17	214572	5.46	K.DIVLLLFMR.L + Oxidation (M)
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	17	67527	9.56	K.AQINVALKIIR.N

Appendix Table A26 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268553945	C. briggsae CBR-TAG-313 protein [Caenorhabditis briggsae]	17	15661	9.62	R.QTFQSAVDLK.Q
gi|268557190	Hypothetical protein CBG23278 [Caenorhabditis briggsae]	17	148936	9.09	R.NDVEGNVMDK.G + Oxidation (M)
gi|291220888	PREDICTED: tyrosine-protein phosphatase non-receptor type, putative-like [Saccoglossus kowalevskii]	17	120017	8.75	R.QTVAFSLKDK.D
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	17	44167	8.9	K.MPGPTVVPIR.E + Oxidation (M)
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	17	20283	7.79	R.GNGGGGGGGGGSR.G

Appendix Table A27 Protein identification data form LCMS analysis. This table presented data of gel number 9 (G9) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1468981	angiotensin-converting enzyme-like protein [Rhipicephalus microplus]	32	75495	5.84	K.LSNEAASLDGYDNIK.S
gi|242003140	hypothetical protein Phum_PHUM003490 [Pediculus humanus corporis]	29	88309	5.6	K.IISNNKSMR.V + Oxidation (M)
gi|115610675	PREDICTED: similar to ATPase II [Strongylocentrotus purpuratus]	26	133000	5.7	R.NISVESMKR.F + Oxidation (M)
gi|195998363	hypothetical protein TRIADDRAFT_52687 [Trichoplax adhaerens]	26	36979	9.16	K.ILSNNSSESK.E
gi|170065271	conserved hypothetical protein [Culex quinquefasciatus]	26	48249	6.24	K.LNSTASDSER.T
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	24	48763	5.94	K.LGGEKPAKPK.E
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	24	60310	6.56	K.TYFKIQPK.L
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	24	125426	9.1	R.VGINRLKPK.L
gi|170586404	F-box domain containing protein [Brugia malayi]	24	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	24	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	24	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	24	150695	6.9	K.SCEYIKPK.E
gi|256087458	Collagen alpha-1(V) chain precursor [Schistosoma mansoni]	24	298242	4.54	K.GGDGMPGDRGDTGR.L K.GLPGMPGDPGLPGPK.G + Oxidation (M)
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	23	85695	7.94	K.AATPSPKQPK.E
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	23	112787	4.31	K.LETKGLFMSR.L

Appendix Table A27 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	22	78492	8.75	R.SPELLLQPK.T
gi|86450139	carbamoylphosphate synthetase [Proagomyia sp. JKM-2006]	21	144659	5.53	R.GARVELVPWNHK.L
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	21	23318	10.58	K.ENQKPIKPK.T
gi|268529590	Hypothetical protein CBG21964 [Caenorhabditis briggsae]	21	52705	8.37	-.MVTDVGTRNVVR.L
gi|170588453	oxidoreductase, short chain drogenase/reductase family protein [Brugia yi]	21	44081	6.85	K.GIGKAIAVALIR.R
gi|156400082	predicted protein [Nematostella vectensis]	21	62244	9.78	K.EDKLLDHR.D
gi|241713550	SMC protein, putative [Ixodes scapularis]	20	133226	9.12	-.MHIKSITIDGFK.S + Oxidation (M) / K.QLEETKVNSDIQFTK.D
gi|268577813	C. briggsae CBR-HUM-6 protein [Caenorhabditis briggsae]	20	240868	8.88	K.DSGNENDSTDSSR.R
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	20	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	19	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	19	194679	8.93	R.RVSEGNAPHSK.N
gi|156555682	PREDICTED: similar to CG32133-PA [Nasonia vitripennis]	19	177790	9.03	K.HPNVVQALREGK.R
gi|156363128	predicted protein [Nematostella vectensis]	18	37562	6.31	K.FPVRPLIIK.T
gi|72092147	PREDICTED: similar to DEAH (Asp-Glu-Ala-His) box polypeptide 29 [Strongylocentrotus purpuratus]	18	94019	9.28	R.LLLNMFLEK.K
gi|256081605	hypothetical protein [Schistosoma mansoni]	18	93250	4.97	K.IILNMFLEK.L

Appendix Table A27 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170593295	Human spg [Brugia malayi]	18	99395	7.89	R.NLVDTAYKR.T
gi|156364491	predicted protein [Nematostella vectensis]	18	68209	5.6	K.DQVGQVNPPK.Y
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	18	225555	5.77	K.NLTLTFKSR.A
gi|289740305	hypothetical protein [Glossina morsitans morsitans]	18	36626	9.97	R.NLLTDTFQK.D
gi|256075911	proline-serine-threonine phosphatase interacting protein [Schistosoma mansoni]	17	142156	6.75	R.VDMGQRLFNK.L + Oxidation (M)
gi|256085723	protein kinase [Schistosoma mansoni]	17	211517	6.78	R.LSAAPVLLDENGLPVR.L
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	17	6635	9.81	K.TITDNKEMK.T
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	17	34876	7.64	R.RGTDNAAVFK.F
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	17	34737	8.06	K.IINLAQGKPK.G
gi|122103768	RecName: Full=60S ribosomal protein L17	17	21891	10.81	R.VVAKPREDEPHK.K
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	17	66947	8.64	K.ILKNLAGKPK.I
gi|158284702	AGAP003271-PA [Anopheles gambiae str. PEST]	17	525812	5.89	K.TSIIDFTVTMRGLEDQLLGR.V + Oxidation (M)
gi|170030867	histone h3 methyltransferase [Culex quinquefasciatus]	17	187209	9.26	R.EAPMAPHLMDDRMDR.L + 2 Oxidation (M)
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	17	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|170065497	cd36 antigen [Culex quinquefasciatus]	17	55347	5.04	R.NISKTEIFK.V

Appendix Table A27 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170592993	hypothetical protein Bm1_48925 [Brugia malayi]	17	16445	8.48	K.LLSKMAEDR.D + Oxidation (M)
gi|170053186	tubulin beta-3 chain [Culex quinquefasciatus]	17	51653	6.1	K.LLTNMVPYK.K
gi|156366213	predicted protein [Nematostella vectensis]	17	56054	8.98	K.GAAGSRPPSPER.D
gi|157813252	putative histidyl-tRNA synthetase [Mastigoproctus giganteus]	17	17103	4.8	K.MAKEGLEAMK.L + 2 Oxidation (M)
gi|122937787	AAEL000054-PA [Aedes aegypti]	16	385687	6.12	K.NKAAAAAAAASVPQSSGDSSGTNSGGK.V

Appendix Table A28 Protein identification data form LCMS analysis. This table presented data of gel number 10 (G10) which found protein bands in tick's gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	41	23318	10.58	K.ENQKPIKPK.T
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	28	34737	8.06	K.IINLAQGKPK.G
gi|158287207	AGAP011349-PA [Anopheles gambiae str. PEST]	27	65162	9.04	R.SERLPQQPK.V
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	27	66947	8.64	K.ILKNLAGKPK.I
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	26	75672	9.35	K.TLTFEEIRK.K
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	23	24881	9.76	K.RADPGIFAWEIR.D
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	23	126171	7.14	R.IITLLRQIIR.T
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	22	165556	6.1	K.MVLPRLQIIR.G
gi|170574856	Protein kinase domain containing protein [Brugia malayi]	22	26666	9.93	R.DLDHNDQLLR.I
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	22	110364	5.32	K.MNPFNVPK.T
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	22	67527	9.56	K.AQINVALKIIR.N
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	21	20283	7.79	R.GNGGGGGGGGSR.G
gi|156363128	predicted protein [Nematostella vectensis]	21	37562	6.31	K.FPVRPLIIK.T
gi|241608961	hypothetical protein IscW_ISCW019269 [Ixodes scapularis]	21	15145	10.5	K.DGGLPPAVHFGAEARR.R
gi|1835720	hexamerin 2 alpha [Aedes aegypti]	20	82016	5.75	-.EDKITYADK.D

Appendix Table A28 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|91088505	PREDICTED: similar to melanin-inhibiting protein [Tribolium castaneum]	20	40857	4.78	K.DLASLPLKPK.A
gi|221130433	PREDICTED: similar to thioredoxin domain containing 11 [Hydra magnipapillata]	20	61790	9.12	R.LINRVNKPK.I
gi|241999176	neurofilament medium polypeptide, putative [Ixodes scapularis]	20	58842	5.1	K.QMPKPKKPK.K
gi|268555432	Hypothetical protein CBG22444 [Caenorhabditis briggsae]	20	137733	5.77	R.KPEKLLEPK.L
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	19	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|535593	histone H1-II-1 [Chironomus thummi]	19	24783	10.65	K.KPVAEKKPK.A
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	19	121129	8.33	K.GGGGGGGGGGGDK.K
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	18	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|170590480	60S ribosomal protein L44 [Brugia malayi]	18	67827	9.66	R.KVSDNIMEK.L + Oxidation (M)
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	18	165126	9.32	R.ILLDQRVGAPR.Y
gi|156541190	PREDICTED: similar to signal recognition particle receptor alpha subunit [Nasonia vitripennis]	18	69406	8.39	K.KQNKPIEVPK.V
gi|242007573	conserved hypothetical protein [Pediculus humanus corporis]	17	42427	8.61	K.MIQVMFEK.Y
gi|196004921	hypothetical protein TRIADDRAFT_56227 [Trichoplax adhaerens]	17	15043	7.07	K.LLVEHGGDVNK.G
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	17	58245	8.67	K.VRYYQTPVR.D
gi|256052137	hypothetical protein [Schistosoma mansoni]	17	102409	7.36	K.KMIVNPVPK.K

Appendix Table A28 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|4104390	ATP synthase F0 subunit 8 [Tetilla sp.]	17	7593	9.52	K.LETILII.-
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	17	48812	8.57	K.SQKYGHVK.S
gi|221122797	PREDICTED: similar to Ift46 protein [Hydra magnipapillata]	17	60790	5.47	R.NNDGFLYNPK.D
gi|256086074	rna polymerase III (DNA directed) 39kD subunit-related [Schistosoma mansoni]	17	34859	6.47	K.MIKEVPMAVGHK.Q + Oxidation (M)
gi|29841276	hypothetical protein [Schistosoma japonicum]	16	25838	4.11	K.IKMCMSNFHLPVSR.Y + Oxidation (M)
gi|4218957	cytoplasmic polyadenylation element-binding protein [Spisula solidissima]	16	74011	8.13	R.NTKGHTNLALL.-
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	16	115140	8.72	K.MGPPMQGR.G + Oxidation (M)
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	16	47623	7.81	R.LLVASQPEVLI.-
gi|115685900	PREDICTED: similar to sorbitol dehydrogenase, partial [Strongylocentrotus purpuratus]	16	35925	6.19	K.RAGVTIGSK.V
gi|242007326	conserved hypothetical protein [Pediculus humanus corporis]	16	130065	8.43	K.DLVHEMVLK.K + Oxidation (M)
gi|268535718	Hypothetical protein CBG21760 [Caenorhabditis briggsae]	15	120295	10.17	R.LLSSSRPPPAR.S
gi|170574417	KH domain containing protein [Brugia malayi]	15	142287	6.2	K.VEIPGEYHR.T
gi|118783458	AGAP004126-PA [Anopheles gambiae str. PEST]	15	24643	9.64	K.SSISLGDSFELMK.T + Oxidation (M)
gi|115739527	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	15	113994	6.2	R.MPPHLMMDK.G
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	15	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)

Appendix Table A28 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|158288833	AGAP000430-PA [Anopheles gambiae str. PEST]	15	64765	8.81	R.LAAGAAPARLDGR.L
gi|91081321	PREDICTED: similar to CG5198 CG5198-PA [Tribolium castaneum]	15	36861	4.72	K.MLNYMKPK.E
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	15	85695	7.94	K.AATPSPKQPK.E
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	15	40519	6.4	K.EGGPPKSKPK.K
gi|156383423	predicted protein [Nematostella vectensis]	15	11177	5.05	R.SIDDFWTGVP.-

Appendix Table A29 Protein identification data form LCMS analysis. This table presented data of gel number 10 (G10) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	43	42046	8.57	-.MAALTKGVFIVAAK.R
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	38	34737	8.06	K.IINLAQGKPK.G
gi|34500282	Bm95 protein [Rhipicephalus microplus]	34	67154	6.44	K.ANCQCPPDTKPGEIGCIER.T
gi|157125808	membrane traffic protein [Aedes aegypti]	26	51628	8.84	R.MAALVCIPQTTSK.R
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	24	23318	10.58	K.ENQKPIKPK.T
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	24	66947	8.64	K.ILKNLAGKPK.I
gi|91095277	PREDICTED: similar to Histone H2B [Tribolium castaneum]	24	18533	10.43	R.DEGPMVPIPK.V
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	22	20283	7.79	R.GNGGGGGGGGGSR.G
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	22	225555	5.77	K.DQVGQVNPPK.Y
gi|115659	RecName: Full=Short-chain collagen C4	22	36445	4.58	K.GDTGAPGPQGPK.G
gi|156540708	PREDICTED: similar to tyrosine recombinase [Nasonia vitripennis]	22	62808	8.95	K.NQTKLLLSLYK.E
gi|3892088	prophenoloxidase [Anopheles gambiae]	21	78684	6.3	K.FTVTLNAGANTIVR.R
gi|158299477	AGAP008858-PA [Anopheles gambiae str. PEST]	21	146743	5.7	R.ARNSPAPSPGK.L
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	21	16163	9.54	-.MAALLLQLNSCSR.K
gi|156363128	predicted protein [Nematostella vectensis]	21	37562	6.31	K.FPVRPLIIK.T

Appendix Table A29 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	20	165126	9.32	R.ILLDQRVGAPR.Y
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	20	9092	9.51	-.MECLRSVLTIAGK.A
gi|115613197	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	20	76899	9.19	K.DVGNVTFASVK.T
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	19	115140	8.72	K.MGPPMQGR.G + Oxidation (M)
gi|221130433	PREDICTED: similar to thioredoxin domain containing 11 [Hydra magnipapillata]	19	61790	9.12	R.LINRVNKPK.I
gi|170039003	conserved hypothetical protein [Culex quinquefasciatus]	18	11130	6.08	K.LLETMKR.C + Oxidation (M)
gi|170590494	hypothetical protein Bm1_42720 [Brugia malayi]	18	33592	9.41	R.ILETESSK.E
gi|193690530	PREDICTED: similar to predicted protein, partial [Acyrthosiphon pisum]	18	21838	9.61	K.ILETFRK.T
gi|196016025	hypothetical protein TRIADDRAFT_968 [Trichoplax adhaerens]	18	69008	8.91	K.ILETQFR.L
gi|242014308	endothelin-converting enzyme, putative [Pediculus humanus corporis]	18	78104	6.04	K.LLETTSSR.T
gi|115811968	PREDICTED: similar to Solute carrier family 25, member 36 [Strongylocentrotus purpuratus]	18	155209	9.15	R.YRGLYGGLGTHLVR.Q
gi|158291121	AGAP002345-PA [Anopheles gambiae str. PEST]	18	87465	5.38	R.QCGAIFPHHENMSLLQQQK.L + Oxidation (M)
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	18	121129	8.33	K.GGGGGGGGGGGGDK.K
gi|221131056	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	18	58978	9.45	K.DEGAFIRFK.K
gi|196008973	hypothetical protein TRIADDRAFT_58074 [Trichoplax adhaerens]	17	165208	6.49	K.TDPENSLAIHMQTAINTDSLK.S

Appendix Table A29 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170052375	conserved hypothetical protein [Culex quinquefasciatus]	17	48084	6.85	M.LSLDNFSVETYGMYR.I + Oxidation (M)
gi|156544936	PREDICTED: hypothetical protein [Nasonia vitripennis]	17	18225	7.88	R.MPTGPDGTKGFNVR.R
gi|268536920	Hypothetical protein CBG05472 [Caenorhabditis briggsae]	17	37769	8.85	K.GGTGFGVMDLLNQTVSQSIFGPR.R
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	17	126171	7.14	R.IITLLRQIIR.T
gi|78271921	vitellogenin [Bombyx mandarina]	17	203420	6.99	R.MRDMPIVTMAPGALSFYQPLK.A + Oxidation (M)
gi|268579695	Hypothetical protein CBG14851 [Caenorhabditis briggsae]	16	115204	5.96	K.ILETKMR.Q + Oxidation (M)
gi|66557857	PREDICTED: similar to fat2 CG7749-PA, isoform A isoform 2, partial [Apis mellifera]	16	130998	4.87	R.VMVDDVNDNAPSFALPSYTVK.I + Oxidation (M)
gi|268580415	C. briggsae CBR-PPK-3 protein [Caenorhabditis briggsae]	16	170200	5.58	R.EVDGTFMKVR.E
gi|256076662	venom allergen-like (VAL) 22 protein [Schistosoma mansoni]	16	25779	9.55	K.LLVLHRR.F
gi|221132241	PREDICTED: similar to zinc finger protein Gli2 [Hydra magnipapillata]	16	187514	9.23	K.ILSNAVHNNYIAGLRR.I
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	16	75672	9.35	K.TLTFEEIRK.K
gi|221118628	PREDICTED: similar to AGAP010295-PA [Hydra magnipapillata]	16	79436	8.87	K.LLVMKFR.K
gi|241117506	bone morphogenetic protein, putative [Ixodes scapularis]	16	47232	4.47	R.ILVMRFK.V
gi|284005204	period [Acyrthosiphon pisum]	16	117030	6.03	K.NCQLTQKMLLR.H + Oxidation (M)
gi|15127838	high voltage-activated calcium channel Cav1 [Schistosoma mansoni]	16	205261	7.05	K.DGDDQK.D

Appendix Table A29 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|19070220	6-phosphogluconate dehydrogenase [Musculium securis]	16	19978	6.21	R.DTADQK.G
gi|66520061	PREDICTED: similar to Transcription elongation factor S-II (RNA polymerase II elongation factor DMS-II) (TFIIS) [Apis mellifera]	16	35553	8.93	K.DDGDQK.K
gi|72006277	PREDICTED: similar to TAF6-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor [Strongylocentrotus purpuratus]	16	42759	6.33	K.ATDDKK.Y
gi|196007044	predicted protein [Trichoplax adhaerens]	16	55071	9.73	R.ASEDKK.L
gi|221112147	PREDICTED: similar to H0525C06.10, partial [Hydra magnipapillata]	16	54447	9.35	K.DGDDKK.V

Appendix Table A30 Protein identification data form LCMS analysis.This table presented data of gel number 10 (G10) which found protein bands in Bm86 proteins.

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	33	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	35	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|170046628	U3 small nucleolar ribonucleoprotein protein MPP10 [Culex quinquefasciatus]	27	75364	4.67	K.LLEKGIKPK.G
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	27	75672	9.35	K.TLTFEEIRK.K
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	25	112787	4.31	K.LETKGLFMSR.L
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	25	24881	9.76	K.RADPGIFAWEIR.D
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	24	48763	5.94	K.LGGEKPAKPK.E
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	24	60310	6.56	K.TYFKIQPK.L
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	24	38467	9.67	K.IKSNPIQPK.F
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	24	150695	6.9	K.SCEYIKPK.E
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	23	85695	7.94	K.AATPSPKQPK.E
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	23	194679	8.93	R.RVSEGNAPHSK.N
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	22	78492	8.75	R.SPELLLQPK.T
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	21	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|156400082	predicted protein [Nematostella vectensis]	21	62244	9.78	K.EDKLLDHR.D

Appendix Table A30 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|118781274	Toll-like receptor (AGAP010669-PA) [Anopheles gambiae str. PEST]	21	116352	6.2	K.DVQTTDNMR.M
gi|115647211	PREDICTED: similar to MGC80611 protein [Strongylocentrotus purpuratus]	21	23945	5.48	K.ELTAGLSQYNK.S
gi|78190747	succinate dehydrogenase iron-sulfur protein [Aphrocallistes vastus]	20	11439	8.15	R.INTDSSKVSK.I
gi|156391805	predicted protein [Nematostella vectensis]	19	23211	4.12	R.DVSAADKSTSR.N
gi|195999698	predicted protein [Trichoplax adhaerens]	18	58417	8.96	R.NVTEYLLAR.L
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	18	10796	6.08	K.GVVGTIVVNGEGIPIK.S
gi|72167364	PREDICTED: similar to aquaporin 4, partial [Strongylocentrotus purpuratus]	18	33778	4.77	K.DDLGSADFWR.A
gi|242017225	DNA polymerase epsilon, catalytic subunit A, putative [Pediculus humanus corporis]	18	261671	6.5	K.LIDLKNNPVR.N
gi|189237783	PREDICTED: similar to 2-phosphodiesterase [Tribolium castaneum]	17	65715	8.33	R.DKSTWVQVANTFR.Y
gi|290563030	Cyclin-G2 [Lepeophtheirus salmonis]	17	38213	8.22	K.LRPTLPTIKESGQANVQR.R
gi|221106965	PREDICTED: similar to CG31809 CG31809-PB, partial [Hydra magnipapillata]	17	28677	7.74	K.LNVLLICKNTDK.L
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	17	6635	9.81	K.TITDNKEMK.T
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	17	34876	7.64	R.RGTDNAAVFK.F
gi|268580415	C. briggsae CBR-PPK-3 protein [Caenorhabditis briggsae]	17	170200	5.58	R.EVDGTFMKVR.E
gi|56462288	protease inhibitor 5 [Lonomia obliqua]	17	10165	6.08	R.DTVTGACVEK.C

Appendix Table A30 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	16	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|170063838	cytochrome P450 6B5 [Culex quinquefasciatus]	16	57010	8.93	K.DVAMKFNVR.L
gi|242024928	hypothetical protein Phum_PHUM603300 [Pediculus humanus corporis]	16	295267	9.04	K.VDYTILEKR.E
gi|91076832	PREDICTED: similar to GA20540-PA [Tribolium castaneum]	16	145934	7.49	R.VQSEFDQVK.L
gi|157111532	f-box protein [Aedes aegypti]	15	67264	9.67	R.NDVSPALEVAPITPK.R
gi|72152487	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	15	30760	6.23	K.NLPDINVRYAVR.G
gi|91091226	PREDICTED: hypothetical protein [Tribolium castaneum]	15	23847	7.93	K.ISDGVHLVSVR.S
gi|156383423	predicted protein [Nematostella vectensis]	15	11177	5.05	R.SIDDFWTGVP.-
gi|91079981	PREDICTED: similar to AGAP005961-PA [Tribolium castaneum]	15	91409	6.59	K.GTGKQLHDAVR.V
gi|211938970	Chain A, A Non-Cytotoxic Dsred Variant For Whole-Cell Labeling	15	24965	7.28	K.SIYMAKKPVK.L + Oxidation (M)
gi|34610107	embryo cathepsin L-associated protein [Artemia franciscana]	15	37064	6.84	K.EVLRYHIAR.G
gi|183179970	LAM-2 [Caenorhabditis remanei]	15	32936	4.52	-.TAGVAPSASDPK.Q
gi|158293448	AGAP008690-PA [Anopheles gambiae str. PEST]	15	63872	9.59	K.VLLLNGAFQDGRK.L
gi|268569090	Hypothetical protein CBG08481 [Caenorhabditis briggsae]	15	39310	4.88	R.AVAGEFQPPLIAPK.L
gi|66521724	PREDICTED: similar to Cytochrome P450 6g1 (CYPVIG1) (Cyp6-like protein) [Apis mellifera]	14	50414	8.09	K.TKIPMVLDPK.G + Oxidation (M)

Appendix Table A30 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170040988	serine/threonine-protein kinase D3 [Culex quinquefasciatus]	14	94823	7.8	R.LDSKGHHAGSK.Y
gi|3023865	RecName: Full=Guanine nucleotide-binding protein G(q) subunit alpha; AltName: Full=Guanine nucleotide-binding protein alpha-q	14	41553	5.18	R.INCEIEK.E
gi|29840923	similar to NM_031954 MSTP028 protein in Homo sapiens [Schistosoma japonicum]	14	39450	6.72	R.NIECLEK.Y
gi|156328522	hypothetical protein NEMVEDRAFT_v1g224666 [Nematostella vectensis]	14	51557	7.95	K.ARECLEK.L
gi|157106155	myosin [Aedes aegypti]	14	180581	8.8	K.NLSSDITR.S

Appendix Table A31 Protein identification data form LCMS analysis. This table presented data ofgel number 11 (G11) which found protein bands in tick’s gut proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|193668032	PREDICTED: similar to AGAP007175-PA [Acyrthosiphon pisum]	42	62018	8.64	K.LKGPK.H / K.EDFDSEDDK.N
gi|7141294	xylanase [Meloidogyne incognita]	32	37946	9	K.IKGPK.I
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	31	112787	4.31	K.LETKGLFMSR.L
gi|242023447	conserved hypothetical protein [Pediculus humanus corporis]	31	48829	8.68	K.DFIENSLR.K
gi|268572719	Hypothetical protein CBG13254 [Caenorhabditis briggsae]	31	55921	6.59	K.NLMEITQK.F + Oxidation (M)
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	31	85695	7.94	K.AATPSPKQPK.E
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	31	40519	6.4	K.EGGPPKSKPK.K
gi|158294544	AGAP005651-PA [Anopheles gambiae str. PEST]	30	47687	5.78	R.NIFEDLNK.-
gi|193652752	PREDICTED: similar to exosome complex exonuclease RRP44 [Acyrthosiphon pisum]	29	94487	6.53	M.DFLENLSR.Q
gi|189233623	PREDICTED: similar to fibroblast growth factor receptor [Tribolium castaneum]	26	109725	6.63	K.MIDIETVR.A + Oxidation (M)
gi|256087255	smg-7 (suppressor with morphological effect on genitalia protein 7) [Schistosoma mansoni]	26	170574	6.22	K.NLEFDINK.S
gi|242011525	conserved hypothetical protein [Pediculus humanus corporis]	26	101669	9.74	K.DMIIETKK.K + Oxidation (M)
gi|157134125	hypothetical protein AaeL_AAEL003092 [Aedes aegypti]	24	174099	5.35	R.FDLIEDNK.V
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	24	38467	9.67	K.IKSNPIQPK.F
gi|18920642	trialysin [Triatoma infestans]	23	22658	9.53	K.IKPGK.V

Appendix Table A31 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|115927504	PREDICTED: similar to CG7908-PA [Strongylocentrotus purpuratus]	23	87538	6.01	R.LKPGK.L
gi|195998638	hypothetical protein TRIADDRAFT_21077 [Trichoplax adhaerens]	23	36004	9.91	R.DYNNKVIK.K
gi|242010126	Nuclear RNA export factor, putative [Pediculus humanus corporis]	23	71855	9.43	R.IVYLEDNK.I
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	21	22842	5.43	M.ASLTIPKAPK.K
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	21	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	21	48763	5.94	K.LGGEKPAKPK.E
gi|170586404	F-box domain containing protein [Brugia malayi]	21	53036	9.66	R.QNALLIQPK.A
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	21	138255	6.59	R.GKLIQLQPK.T
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	20	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	20	6635	9.81	K.TITDNKEMK.T
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	20	194679	8.93	R.RVSEGNAPHSK.N
gi|37703935	elongation factor-2 [Eurytemora affinis]	20	79396	5.78	K.LTTXXGKXVK.D
gi|20799320	fasciclin-like protein [Aplysia californica]	20	87461	7	R.INVIDDVMVPPK.G + Oxidation (M)
gi|221121894	PREDICTED: similar to X-ray repair complementing defective repair in Chinese hamster cells 5, partial [Hydra magnipapillata]	19	67312	5.49	K.DLVNPYMQR.Q
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	19	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)

Appendix Table A31 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|78190747	succinate dehydrogenase iron-sulfur protein [Aphrocallistes vastus]	19	11439	8.15	R.INTDSSKVSK.I
gi|66524168	PREDICTED: similar to RhoGEF4 CG8606-PA, isoform A [Apis mellifera]	18	54863	9.23	K.LLLREVLQHTSNK.H
gi|156400082	predicted protein [Nematostella vectensis]	18	62244	9.78	K.EDKLLDHR.D
gi|170049838	4-nitrophenylphosphatase [Culex quinquefasciatus]	18	9770	11.26	-.MIAQDVAFGK.V
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	18	34876	7.64	R.RGTDNAAVFK.F
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	17	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|73402038	ABC transporter ABCH1 [Sarcoptes scabiei type hominis]	17	24807	9.61	K.NISLLSNVSMNVPK.G
gi|268531878	C. briggsae CBR-TRR-1 protein [Caenorhabditis briggsae]	17	474950	6.3	R.DLEMIGYTAK.M + Oxidation (M)
gi|196015607	predicted protein [Trichoplax adhaerens]	16	31694	5.17	K.DIEVAFNSLK.F
gi|170596587	3'-5' exonuclease family protein [Brugia malayi]	16	81434	6.94	R.AGTVESFAAKR.A
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	16	56983	9.9	-.TLTHRVR.L
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	16	65748	9.67	R.TITHSPVK.S
gi|262302289	RNA polymerase subunit 1 [Semibalanus balanoides]	16	27138	5.91	K.DNVYCTTYT.-
gi|121484216	cuticle protein CB6 [Portunus pelagicus]	16	24116	4.42	K.QTVNYVVEGK.K
gi|170037064	phenylalanyl-tRNA synthetase beta chain [Culex quinquefasciatus]	16	38924	9.08	R.IPKTLPATIHIAR.Q

Appendix Table A31 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170053161	poly [Culex quinquefasciatus]	16	113432	8.9	K.DFGVEYAASGR.A
gi|268577861	Hypothetical protein CBG02177 [Caenorhabditis briggsae]	16	76713	6.36	R.LPNPLLSVAR.E
gi|170029427	o-linked N-acetyl glucosamine transferase [Culex quinquefasciatus]	16	94887	9.3	R.QVPNLAPLPLDPR.K
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	15	20283	7.79	R.GNGGGGGGGGGSR.G
gi|29840983	hypothetical protein [Schistosoma japonicum]	15	36215	5.83	K.TLTHGKPK.C

Appendix Table A32 Protein identification data form LCMS analysis. This table presented data of gel number 11 (G11) which found protein bands in Bm95 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|115659	RecName: Full=Short-chain collagen C4	47	36445	4.58	K.GDTGAPGPQGPK.G
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	43	42046	8.57	-.MAALTKGVFIVAAK.R
gi|196005577	hypothetical protein TRIADDRAFT_56895 [Trichoplax adhaerens]	31	72241	9.49	K.NDVLSSVMK.A + Oxidation (M)
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	28	165556	6.1	K.MVLPRLQIIR.G
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	28	85695	7.94	K.AATPSPKQPK.E
gi|66507736	PREDICTED: similar to Elongin B CG4204-PA [Apis mellifera]	27	13138	4.85	K.DNVLMSDSK.F
gi|170051303	aminopeptidase N [Culex quinquefasciatus]	27	104069	5.9	R.AASVLNMFR.Q
gi|170586382	DEAH [Brugia malayi]	27	77562	9.2	K.KTVLNMFR.D
gi|240270422	serpin 10 plasmodium-related inhibitory serine protease inhibitor [Anopheles gambiae]	25	24471	5.25	K.QTVIDNYR.R
gi|195999142	hypothetical protein TRIADDRAFT_21036 [Trichoplax adhaerens]	24	21611	9.92	K.LDVNDEFR.V
gi|268556740	Hypothetical protein CBG08656 [Caenorhabditis briggsae]	24	46743	5.75	-.DQIKMASEWGYNQEDIIAALK.E + Oxidation (M)
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	24	44167	8.9	K.MPGPTVVPIR.E + Oxidation (M)
gi|170048915	conserved hypothetical protein [Culex quinquefasciatus]	23	46121	7	R.DALQMSSEMVLLDLLASWSR.E + Oxidation (M)
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	22	67527	9.56	K.AQINVALKIIR.N
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	22	48763	5.94	K.LGGEKPAKPK.E

Appendix Table A32 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|157125808	membrane traffic protein [Aedes aegypti]	22	51628	8.84	R.MAALVCIPQTTSK.R
gi|21425408	metalloprotease III [Ostertagia ostertagi]	22	57778	6.77	R.MPVIMFNR.S
gi|241155753	excitatory amino acid transporter, putative [Ixodes scapularis]	22	102695	6.09	K.DIVIRHVR.D
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	21	20283	7.79	R.GNGGGGGGGGGSR.G
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	21	125426	9.1	R.VGINRLKPK.L
gi|156550522	PREDICTED: similar to abc transporter [Nasonia vitripennis]	21	78876	8.88	R.VQIIQAKPK.T
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	20	194679	8.93	R.RVSEGNAPHSK.N
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	20	165126	9.32	R.ILLDQRVGAPR.Y
gi|241788743	FGF receptor activating protein, putative [Ixodes scapularis]	20	30361	9.12	R.TGVALHTGPR.L
gi|58613373	qua-1 protein [Caenorhabditis remanei]	20	117928	5.5	K.NGGDDGDDDNGDGNNGGDGDGDDNGK.G
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	20	115140	8.72	K.MGPPMQGR.G + Oxidation (M)
gi|241351505	hypothetical protein IscW_ISCW004874 [Ixodes scapularis]	19	11366	4.77	R.EDPERSGR.G
gi|157110621	hypothetical protein AaeL_AAEL005631 [Aedes aegypti]	19	73987	9.28	K.DNIGGFPVK.L
gi|91081233	PREDICTED: similar to hormone-sensitive lipase [Tribolium castaneum]	19	84776	5.09	K.YKSKPPAR.G
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	19	22842	5.43	M.ASLTIPKAPK.K

Appendix Table A32 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	19	1009	6.48	-.MPQLSPHSL.- + Oxidation (M)
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	19	138255	6.59	R.GKLIQLQPK.T
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	19	38467	9.67	K.IKSNPIQPK.F
gi|118781575	AGAP010405-PA [Anopheles gambiae str. PEST]	18	17503	9.32	K.TAQSMMRPK.S + 2 Oxidation (M)
gi|170047092	serrate protein [Culex quinquefasciatus]	18	419035	5.51	R.IRLGCTAR.Y
gi|268554292	C. briggsae CBR-MYO-5 protein [Caenorhabditis briggsae]	18	219524	5.96	R.KVEGDLQHLQSEVEEALSDAK.V
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	17	174650	5.27	R.GAPGLSGLKGPK.G
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	17	58245	8.67	K.VRYYQTPVR.D
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	17	126171	7.14	R.IITLLRQIIR.T
gi|170574856	Protein kinase domain containing protein [Brugia malayi]	17	26666	9.93	R.DLDHNDQLLR.I
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	17	121129	8.33	K.GGGGGGGGGGGDK.K
gi|268537058	C. briggsae CBR-DPY-26 protein [Caenorhabditis briggsae]	17	141467	4.66	K.ATDAWTNYDVVKMIVNR.D
gi|193654823	PREDICTED: similar to N-myristoyltransferase, putative [Acyrthosiphon pisum]	17	54093	8.94	M.SETPNIPDDHQTNTELNQTK.K
gi|241146856	conserved hypothetical protein [Ixodes scapularis]	17	14220	6.1	R.CLEQNSWDLQK.A
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	17	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)

Appendix Table A32 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	17	376559	5.9	K.LMQLMVMK.H + 2 Oxidation (M)
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	17	66947	8.64	K.ILKNLAGKPK.I
gi|158284931	AGAP002202-PA [Anopheles gambiae str. PEST]	16	26868	6.79	R.LTTDIPYEK.M
gi|110755884	PREDICTED: similar to zinc finger protein 93 homolog [Apis mellifera]	16	48460	9.51	-.MLVLVPRVR.A
gi|156364402	predicted protein [Nematostella vectensis]	16	77316	5.65	K.VDAPNLDAEGAFIR.S

Appendix Table A33 Protein identification data form LCMS analysis. This table presented of form gel number 11 (G11) which found protein bands in Bm86 proteins

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|170068361	conserved hypothetical protein [Culex quinquefasciatus]	31	140481	6.91	K.EPEPTPELPK.R
gi|158291121	AGAP002345-PA [Anopheles gambiae str. PEST]	25	87465	5.38	R.QCGAIFPHHENMSLLQQQK.L + Oxidation (M)
gi|221122805	PREDICTED: similar to predicted protein [Hydra magnipapillata]	24	110792	8.98	R.ERPTINKPKR.D
gi|156547031	PREDICTED: similar to B lymphoma Mo-MLV insertion region (mouse) [Nasonia vitripennis]	23	158686	9.36	K.QSLSNSNMELESKDNEQSIK.R
gi|256089948	lupus la ribonucleoprotein [Schistosoma mansoni]	23	111137	8.27	R.LALEDEASGYR.Y / R.DELDVGFAFDLSARPSARTTK.G
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	22	126171	7.14	R.IITLLRQIIR.T
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	22	194679	8.93	R.RVSEGNAPHSK.N
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	22	122944	8.75	R.DVKILMGMNK.D + 2 Oxidation (M)
gi|196001089	hypothetical protein TRIADDRAFT_13672 [Trichoplax adhaerens]	21	51867	7.75	K.ILQNLILEPVK.D
gi|154424522	cytochrome oxidase subunit I [Lamprodrilus wagneri]	21	22695	5.82	-.LCFITGVWAGMIGAGMSLLIR.V + Oxidation (M)
gi|221124258	PREDICTED: similar to Signal recognition particle protein 54k CG4659-PA [Hydra magnipapillata]	21	44795	9.43	K.GGGMMKMMK.R + 2 Oxidation (M)
gi|72057275	PREDICTED: similar to myocardial muscarinic receptor protein [Strongylocentrotus purpuratus]	21	56718	8.25	R.YPGNGSSTNNPAPPSR.V
gi|13775610	putative zinc-metalloproteinase precursor [Glossina morsitans morsitans]	20	29610	4.77	K.DINKLNLMYR.C
gi|110757623	PREDICTED: similar to lethal (2) 01424 CG3845-PB, isoform B [Apis mellifera]	19	105696	8.5	R.GLMDQYFK.R
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	19	6635	9.81	K.TITDNKEMK.T

Appendix Table A33 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	19	34876	7.64	R.RGTDNAAVFK.F
gi|195996053	hypothetical protein TRIADDRAFT_51865 [Trichoplax adhaerens]	19	147604	4.94	K.STHDVAVKLLR.D
gi|270046366	arrestin [Triops granarius]	19	43758	8.99	K.LSLGALAGELVADVPLK.L
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	19	52382	4.11	K.NGGGDDDDDDDEGNGFGRGGGGGR.G
gi|54289293	vitellogenin C1 [Culex quinquefasciatus]	19	242895	5.94	R.CAAVHLLIRAEPQIEMMQR.M + Oxidation (M)
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	19	129521	6.67	R.LTTSMILER.L + Oxidation (M)
gi|110759005	PREDICTED: similar to CG11148-PA, isoform A isoform 1 [Apis mellifera]	18	147334	7.34	R.LLQNQQLLLR.Q
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	18	165556	6.1	K.MVLPRLQIIR.G
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	18	67527	9.56	K.AQINVALKIIR.N
gi|110764460	PREDICTED: similar to CG6525-PA [Apis mellifera]	18	256867	5.84	K.AMGQQMEEKGIEWVCPNCAK.K + Oxidation (M)
gi|156386258	predicted protein [Nematostella vectensis]	18	460607	5.42	K.GDLDYESRQR.F
gi|15298096	green fluorescent protein [Montastraea cavernosa]	17	26002	6.09	K.LRMQGVVNGHK.F
gi|157123358	hypothetical protein AaeL_AAEL000237 [Aedes aegypti]	17	41639	9.35	K.KPKPSGGGGGGGAPR.G
gi|115911499	PREDICTED: similar to laminin A chain, putative [Strongylocentrotus purpuratus]	17	61148	4.15	R.DLNPGKEAAEEEK.R
gi|4218957	cytoplasmic polyadenylation element-binding protein [Spisula solidissima]	17	74011	8.13	R.NTKGHTNLALL.-

Appendix Table A33 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|110772653	PREDICTED: similar to asparaginyl-tRNA synthetase 2 (mitochondrial)(putative), partial [Apis mellifera]	17	43455	5.1	K.LETAAQGNYTR.L
gi|156317820	hypothetical protein NEMVEDRAFT_v1g225554 [Nematostella vectensis]	17	41873	5.08	K.GISDVAAAVAK.T
gi|221122797	PREDICTED: similar to Ift46 protein [Hydra magnipapillata]	16	60790	5.47	R.NNDGFLYNPK.D
gi|3183022	RecName: Full=50 kDa hatching enzyme; Short=HEZ; Short=HE; AltName: Full=Envelysin; AltName: Full=Sea-urchin-hatching proteinase; Contains: RecName: Full=38 kDa hatching enzyme; Contains: RecName: Full=32 kDa hatching enzyme non-specific; Contains: RecName: Full=15 kDa peptide; Flags: Precursor	16	66485	5.24	R.DDIAGITSLYGR.N
gi|37703935	elongation factor-2 [Eurytemora affinis]	16	79396	5.78	K.LTTXXGKXVK.D
gi|115634798	PREDICTED: similar to ENSANGP00000007239 [Strongylocentrotus purpuratus]	15	305747	5.9	R.EKHDSSPGILR.Q
gi|189236109	PREDICTED: similar to nucleoporin 37 [Tribolium castaneum]	15	36446	5.08	K.LLIAEKIGLIR.F
gi|256087557	hypothetical protein [Schistosoma mansoni]	15	10720	4.72	K.LTYQLMNIQSYEK.Q + Oxidation (M)
gi|170574231	Type I phosphodiesterase / nucleotide pyrophosphatase family protein [Brugia malayi]	15	55457	4.78	R.SDELKQALINVDLSLLLLQQK.L
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	15	40358	8.03	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)
gi|6012985	microsomal aminopeptidase [Haemonchus contortus]	15	112731	5.76	K.GLLMLALDR.N
gi|91076832	PREDICTED: similar to GA20540-PA [Tribolium castaneum]	15	145934	7.49	R.VQSEFDQVK.L
gi|156546770	PREDICTED: similar to rap55 [Nasonia vitripennis]	15	61343	9.32	R.NQQPGGAGVKPK.N
gi|268561566	Hypothetical protein CBG18559 [Caenorhabditis briggsae]	15	24210	6.9	R.AITPAYYRGALGILIVYDIAK.H
gi|221091667	PREDICTED: similar to acid phosphatase, prostate long, partial [Hydra magnipapillata]	15	38490	5.34	K.IGMNMTYR.L + Oxidation (M)

Appendix Table A33 (Continued)

Accession number	Protein Hits	Protein score	Mw	PI	Peptide sequence
gi|256081440	tyrosine kinase [Schistosoma mansoni]	15	205589	6.64	K.GIDYLHRK.S
gi|110756688	PREDICTED: similar to CG15786-PA [Apis mellifera]	15	33477	8.83	R.GLMLDPISR.S
gi|289740379	chaperonin complex component TcP-1 zeta subunit [Glossina morsitans morsitans]	15	58847	6.9	R.GLVMDHGTR.H + Oxidation (M)
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	15	47623	7.81	R.LLVASQPEVLI.-
gi|268531878	C. briggsae CBR-TRR-1 protein [Caenorhabditis briggsae]	15	474950	6.3	R.DLEMIGYTAK.M + Oxidation (M)

Appendix B

The resmblant protein identification lists table presented form gel number 1 (G1) to gel number 11 (G11)

Appendix Table B1 The presence of protein from crude tick's gut protein, Bm95, and Bm86 in gel number 1 (G1).

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	48763	K.LGGEKPAKPK.E	√		√
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	126171	R.IITLLRQIIR.T	√		√
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	406489	K.EPGQLQQLLR.D	√		√
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	10796	K.GVVGTIVVNGEGIPIK.S	√		√
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√		√
gi|156366213	predicted protein [Nematostella vectensis]	56054	K.GAAGSRPPSPER.D	√		√
gi|156400082	predicted protein [Nematostella vectensis]	62244	K.EDKLLDHR.D	√		√
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	165556	K.MVLPRLQIIR.G	√		√
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√		√
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22842	M.ASLTIPKAPK.K	√		√
gi|170059237	conserved hypothetical protein [Culex quinquefasciatus]	117287	K.TLKTIGGYVK.N	√		√
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	1009	-.MPQLSPHSL.- + Oxidation (M)	√		√
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T	√		√
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	6635	K.TITDNKEMK.T	√		√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F	√		√

Appendix Table B1 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	67527	K.AQINVALKIIR.N	√		√
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	34876	R.RGTDNAAVFK.F	√		√
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	121129	K.GGGGGGGGGGGGDK.K	√		√
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	376559	K.LMQLMVMK.H + 2 Oxidation (M)	√		√
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	110364	K.MNPFNVPK.T	√		√
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	129521	R.LTTSMILER.L + Oxidation (M)	√		√
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)		√	√

Appendix Table B2 The presence of protein from crude tick's gut protein, Bm95, and Bm86 in gel number 2 (G2).

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	48763	K.LGGEKPAKPK.E	√	√	√
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	406489	K.EPGQLQQLLR.D	√	√	√
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√	√	√
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22842	M.ASLTIPKAPK.K	√	√	√
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T	√	√	√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F	√	√	√
gi|115659	RecName: Full=Short-chain collagen C4	36445	K.GDTGAPGPQGPK.G	√	√	
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G	√	√	
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	115140	K.MGPPMQGR.G + Oxidation (M)	√	√	
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	225555	K.DQVGQVNPPK.Y	√	√	
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I	√	√	
gi|268552739	Hypothetical protein CBG17702 [Caenorhabditis briggsae]	55277	R.DSAITGEQINEDK.I	√	√	
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	376559	K.LMQLMVMK.H + 2 Oxidation (M)	√	√	
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	42046	-.MAALTKGVFIVAAK.R	√	√	
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	22059	M.ENKLDSYR.K	√		√

Appendix Table B2 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	48812	K.SQKYGHVK.S		√	√
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G		√	√
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	99637	K.ALLNGLKAEPR.V		√	√
gi|170047092	serrate protein [Culex quinquefasciatus]	419035	R.IRLGCTAR.Y		√	√
gi|170580994	U1 zinc finger family protein [Brugia malayi]	51370	K.IDGPIEFR.K		√	√
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	1009	-.MPQLSPHSL.- + Oxidation (M)		√	√

Appendix Table B3 The presence of protein from crude tick's gut protein, Bm95, and Bm86 in gel number 3 (G3).

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	10796	K.GVVGTIVVNGEGIPIK.S	√	√	√
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)	√	√	√
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√	√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√	√	
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	9092	-.MECLRSVLTIAGK.A	√	√	
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	16163	-.MAALLLQLNSCSR.K	√	√	
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	40358	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)	√	√	
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	48763	K.LGGEKPAKPK.E	√		√
gi|156400082	predicted protein [Nematostella vectensis]	62244	K.EDKLLDHR.D	√		√
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	22059	M.ENKLDSYR.K	√		√
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22842	M.ASLTIPKAPK.K	√		√
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T	√		√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F	√		√
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	376559	K.LMQLMVMK.H + 2 Oxidation (M)	√		√

Appendix Table B3 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|60691514	SJCHGC05193 protein [Schistosoma japonicum]	9688	R.DKQPNGPLR.S	√		√
gi|156363128	predicted protein [Nematostella vectensis]	37562	K.FPVRPLIIK.T		√	√
gi|156366213	predicted protein [Nematostella vectensis]	56054	K.GAAGSRPPSPER.D		√	√
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G		√	√
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	225555	K.DQVGQVNPPK.Y		√	√

Appendix Table B4 The presence of protein from crude tick’s gut protein, Bm95, and Bm86 in gel number 4 (G4).

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)	√	√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G	√	√	√
gi|115659	RecName: Full=Short-chain collagen C4	36445	K.GDTGAPGPQGPK.G	√	√	
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I	√		√
gi|115749236	PREDICTED: similar to BIG3, partial [Strongylocentrotus purpuratus]	169014	K.MEAAHIKGIIR.A		√	√
gi|15298096	green fluorescent protein [Montastraea cavernosa]	26002	K.LRMQGVVNGHK.F		√	√
gi|170047320	gp210 [Culex quinquefasciatus]	206844	R.NMIYENGPER.I + Oxidation (M)		√	√
gi|189236109	PREDICTED: similar to nucleoporin 37 [Tribolium castaneum]	36446	K.LLIAEKIGLIR.F		√	√
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	174650	R.GAPGLSGLKGPK.G		√	√
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	58116	R.YHQKPLAPK.V		√	√

Appendix Table B5 The presence of protein from crude tick's gut protein, Bm95, and Bm86 in gel number 5 (G5)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)	√	√	
gi|15298096	green fluorescent protein [Montastraea cavernosa]	26002	K.LRMQGVVNGHK.F	√	√	
gi|170583347	hypothetical protein Bm1_25390 [Brugia malayi]	22336	K.TPVTPKQIVLK.N	√	√	
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	52382	K.NGGGDDDDDDDEGNGFGRGGGGGR.G	√	√	
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	126171	R.IITLLRQIIR.T	√		√
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	10796	K.GVVGTIVVNGEGIPIK.S	√		√
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√		√
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	48812	K.SQKYGHVK.S	√		√
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√		√
gi|170047092	serrate protein [Culex quinquefasciatus]	419035	R.IRLGCTAR.Y	√		√
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	58245	K.VRYYQTPVR.D	√		√
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	121129	K.GGGGGGGGGGGGDK.K	√		√
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	110364	K.MNPFNVPK.T	√		√

Appendix Table B5 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|91079981	PREDICTED: similar to AGAP005961-PA [Tribolium castaneum]	91409	K.GTGKQLHDAVR.V	√		√
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	48763	K.LGGEKPAKPK.E		√	√
gi|156400082	predicted protein [Nematostella vectensis]	62244	K.EDKLLDHR.D		√	√
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T		√	√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F		√	√
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	40519	K.EGGPPKSKPK.K		√	√
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	9092	-.MECLRSVLTIAGK.A		√	√
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	16163	-.MAALLLQLNSCSR.K		√	√

Appendix Table B6 The presence of protein from crude tick’s gut protein, Bm95, and Bm86 in gel number 6 (G6)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√	√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√	√	√
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	121129	K.GGGGGGGGGGGGDK.K	√	√	√
gi|15298096	green fluorescent protein [Montastraea cavernosa]	26002	K.LRMQGVVNGHK.F	√	√	
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G	√	√	
gi|170592699	ABC transporter family protein [Brugia malayi]	253101	R.DNYLIDLK.Y	√	√	
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	67527	K.AQINVALKIIR.N	√	√	
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	52382	K.NGGGDDDDDDDEGNGFGRGGGGGR.G	√	√	
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	75672	K.TLTFEEIRK.K	√	√	
gi|256078034	initiation factor 5a [Schistosoma mansoni]	17855	K.DYQLVDIK.D	√	√	
gi|37703935	elongation factor-2 [Eurytemora affinis]	79396	K.LTTXXGKXVK.D	√	√	
gi|156363128	predicted protein [Nematostella vectensis]	37562	K.FPVRPLIIK.T	√		√
gi|170580994	U1 zinc finger family protein [Brugia malayi]	51370	K.IDGPIEFR.K	√		√
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I	√		√

Appendix Table B6 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	23318	K.ENQKPIKPK.T	√		√
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	129521	R.LTTSMILER.L + Oxidation (M)	√		√
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	165126	R.ILLDQRVGAPR.Y	√		√
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	K.VKGQSKPK.G R.DVKILMGMNK.D + 2 Oxidation (M)		√	√
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	48812	K.SQKYGHVK.S		√	√

Appendix Table B7 The presence of protein from crude tick's gut protein, Bm95, and Bm86 in gel number 7 (G7)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√	√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I	√	√	√
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	174650	R.GAPGLSGLKGPK.G	√	√	√
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	101408	K.KATEYGSTPK.W	√	√	√
gi|157120630	hypothetical protein AaeL_AAEL009087 [Aedes aegypti]	31539	R.YHMVIHGPK.K	√	√	
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	406489	K.EPGQLQQLLR.D	√		√
gi|115659	RecName: Full=Short-chain collagen C4	36445	K.GDTGAPGPQGPK.G	√		√
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	85695	K.AATPSPKQPK.E	√		√
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	60310	K.TYFKIQPK.L	√		√
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	99637	K.ALLNGLKAEPR.V	√		√
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	125426	R.VGINRLKPK.L	√		√
gi|170586404	F-box domain containing protein [Brugia malayi]	53036	R.QNALLIQPK.A	√		√
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T	√		√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F	√		√

Appendix Table B7 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	150695	K.SCEYIKPK.E	√		√
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	78492	R.SPELLLQPK.T	√		√
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G		√	√
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	56983	-.TLTHRVR.L		√	√
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	65748	R.TITHSPVK.S		√	√
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	58116	R.YHQKPLAPK.V		√	√

Appendix Table B8 The presence of protein from crude tick’s gut protein, Bm95, and Bm86 in gel number 8 (G8)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	406489	K.EPGQLQQLLR.D	√	√	
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√		√
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	121129	K.GGGGGGGGGGGDK.K	√		√
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L		√	√
gi|156400082	predicted protein [Nematostella vectensis]	62244	K.EDKLLDHR.D		√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N		√	√
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G		√	√
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	85695	K.AATPSPKQPK.E		√	√
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	159499	R.DVTETLSTDV.-		√	√
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22842	M.ASLTIPKAPK.K		√	√
gi|196012718	hypothetical protein TRIADDRAFT_60295 [Trichoplax adhaerens]	78546	R.LLDTTTTDAK.E		√	√
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	6635	K.TITDNKEMK.T		√	√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F		√	√
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	40519	K.EGGPPKSKPK.K		√	√
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	34876	R.RGTDNAAVFK.F		√	√

Appendix Table B8 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I		√	√
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	376559	K.LMQLMVMK.H + 2 Oxidation (M)		√	√
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	129521	R.LTTSMILER.L + Oxidation (M)		√	√

Appendix Table B9 The presence of protein from crude tick’s gut protein, Bm95, and Bm86 in gel number 9 (G9)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√	√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G	√	√	√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F	√	√	√
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	126171	R.IITLLRQIIR.T	√	√	
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	22059	M.ENKLDSYR.K	√	√	
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	159499	R.DVTETLSTDV.-	√	√	
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22842	M.ASLTIPKAPK.K	√	√	
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	67527	K.AQINVALKIIR.N	√	√	
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	376559	K.LMQLMVMK.H + 2 Oxidation (M)	√	√	
gi|37703935	elongation factor-2 [Eurytemora affinis]	79396	K.LTTXXGKXVK.D	√	√	
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)	√		√
gi|156363128	predicted protein [Nematostella vectensis]	37562	K.FPVRPLIIK.T	√		√
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I	√		√
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	23318	K.ENQKPIKPK.T	√		√

Appendix Table B9 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	129521	R.LTTSMILER.L + Oxidation (M)	√		√
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	48763	K.LGGEKPAKPK.E		√	√
gi|156400082	predicted protein [Nematostella vectensis]	62244	K.EDKLLDHR.D		√	√
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T		√	√

Appendix Table B10 The presence of protein from crude tick’s gut protein, Bm95, and Bm86 in gel number 10 (G10)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	75672	K.TLTFEEIRK.K	√	√	√
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	126171	R.IITLLRQIIR.T	√	√	
gi|156363128	predicted protein [Nematostella vectensis]	37562	K.FPVRPLIIK.T	√	√	
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G	√	√	
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	115140	K.MGPPMQGR.G + Oxidation (M)	√	√	
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√	√	
gi|221130433	PREDICTED: similar to thioredoxin domain containing 11 [Hydra magnipapillata]	61790	R.LINRVNKPK.I	√	√	
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	121129	K.GGGGGGGGGGGDK.K	√	√	
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I	√	√	
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	23318	K.ENQKPIKPK.T	√	√	
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	165126	R.ILLDQRVGAPR.Y	√	√	
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)	√		√
gi|156383423	predicted protein [Nematostella vectensis]	11177	R.SIDDFWTGVP.-	√		√
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	85695	K.AATPSPKQPK.E	√		√
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	1009	-.MPQLSPHSL.- + Oxidation (M)	√		√
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	24881	K.RADPGIFAWEIR.D	√		√
gi|268580415	C. briggsae CBR-PPK-3 protein [Caenorhabditis briggsae]	170200	R.EVDGTFMKVR.E		√	√

Appendix Table B11 The presence of protein from crude tick’s gut protein, Bm95, and Bm86 in gel number 11 (G11)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)	√	√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	48763	K.LGGEKPAKPK.E	√	√	
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	85695	K.AATPSPKQPK.E	√	√	
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√	√	
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22842	M.ASLTIPKAPK.K	√	√	
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	1009	-.MPQLSPHSL.- + Oxidation (M)	√	√	
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T	√	√	
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F	√	√	
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	376559	K.LMQLMVMK.H + 2 Oxidation (M)	√	√	
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	6635	K.TITDNKEMK.T	√		
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	34876	R.RGTDNAAVFK.F	√		
gi|268531878	C. briggsae CBR-TRR-1 protein [Caenorhabditis briggsae]	474950	R.DLEMIGYTAK.M + Oxidation (M)	√		
gi|37703935	elongation factor-2 [Eurytemora affinis]	79396	K.LTTXXGKXVK.D	√		
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	129521	R.LTTSMILER.L + Oxidation (M)	√		
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	126171	R.IITLLRQIIR.T		√	√
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	165556	K.MVLPRLQIIR.G		√	√
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	67527	K.AQINVALKIIR.N		√	√

Appendix Table B12 Total protein presented in crude tick’s gut protein, Bm95, and Bm86.

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|110758897	PREDICTED: similar to sorting nexin 13 [Apis mellifera]	126171	R.IITLLRQIIR.T	√	√	√
gi|110760097	PREDICTED: similar to CG11321-PA, isoform A [Apis mellifera]	406489	K.EPGQLQQLLR.D	√	√	√
gi|110764754	PREDICTED: similar to roadblock CG10751-PA [Apis mellifera]	10796	K.GVVGTIVVNGEGIPIK.S	√	√	√
gi|115623606	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	122944	R.DVKILMGMNK.D + 2 Oxidation (M)	√	√	√
gi|115659	RecName: Full=Short-chain collagen C4	36445	K.GDTGAPGPQGPK.G	√	√	√
gi|115746571	PREDICTED: similar to SCO-spondin [Strongylocentrotus purpuratus]	112787	K.LETKGLFMSR.L	√	√	√
gi|118781274	Toll-like receptor (AGAP010669-PA) [Anopheles gambiae str. PEST]	116352	K.DVQTTDNMR.M	√	√	√
gi|15298096	green fluorescent protein [Montastraea cavernosa]	26002	K.LRMQGVVNGHK.F	√	√	√
gi|156363128	predicted protein [Nematostella vectensis]	37562	K.FPVRPLIIK.T	√	√	√
gi|156366213	predicted protein [Nematostella vectensis]	56054	K.GAAGSRPPSPER.D	√	√	√
gi|156383423	predicted protein [Nematostella vectensis]	11177	R.SIDDFWTGVP.-	√	√	√
gi|156400082	predicted protein [Nematostella vectensis]	62244	K.EDKLLDHR.D	√	√	√
gi|156541302	PREDICTED: similar to GA21542-PA [Nasonia vitripennis]	194679	R.RVSEGNAPHSK.N	√	√	√
gi|156542082	PREDICTED: similar to epidermal growth factor receptor [Nasonia vitripennis]	165556	K.MVLPRLQIIR.G	√	√	√
gi|156546770	PREDICTED: similar to rap55 [Nasonia vitripennis]	61343	R.NQQPGGAGVKPK.N	√	√	√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|156551159	PREDICTED: similar to DNA-directed RNA polymerase III, 34kD-subunit, putative [Nasonia vitripennis]	34737	K.IINLAQGKPK.G	√	√	√
gi|157130421	hypothetical protein AaeL_AAEL002586 [Aedes aegypti]	48812	K.SQKYGHVK.S	√	√	√
gi|157135755	DEAD box ATP-dependent RNA helicase [Aedes aegypti]	82792	R.KPKQQNGGKPR.F	√	√	√
gi|158284662	Anopheles gambiae str. PEST AGAP012642-PA [Anopheles gambiae str. PEST]	22059	M.ENKLDSYR.K	√	√	√
gi|158287207	AGAP011349-PA [Anopheles gambiae str. PEST]	65162	R.SERLPQQPK.V	√	√	√
gi|158290901	AGAP002502-PA [Anopheles gambiae str. PEST]	115140	K.MGPPMQGR.G + Oxidation (M)	√	√	√
gi|158291265	AGAP003111-PA [Anopheles gambiae str. PEST]	85695	K.AATPSPKQPK.E	√	√	√
gi|158292069	AGAP004349-PA [Anopheles gambiae str. PEST]	20283	R.GNGGGGGGGGGSR.G	√	√	√
gi|158294544	AGAP005651-PA [Anopheles gambiae str. PEST]	47687	R.NIFEDLNK.-	√	√	√
gi|158298901	AGAP009921-PA [Anopheles gambiae str. PEST]	99637	K.ALLNGLKAEPR.V	√	√	√
gi|158299898	AGAP009150-PA [Anopheles gambiae str. PEST]	125426	R.VGINRLKPK.L	√	√	√
gi|170039216	rap guanine nucleotide exchange factor 2 [Culex quinquefasciatus]	159499	K.STHDVAVKLLR.D R.DVTETLSTDV.-	√	√	√
gi|170041554	conserved hypothetical protein [Culex quinquefasciatus]	22842	M.ASLTIPKAPK.K	√	√	√
gi|170047092	serrate protein [Culex quinquefasciatus]	419035	R.IRLGCTAR.Y	√	√	√
gi|170047320	gp210 [Culex quinquefasciatus]	206844	R.NMIYENGPER.I + Oxidation (M)	√	√	√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|170574856	Protein kinase domain containing protein [Brugia malayi]	26666	R.DLDHNDQLLR.I	√	√	√
gi|170580994	U1 zinc finger family protein [Brugia malayi]	51370	K.IDGPIEFR.K	√	√	√
gi|170586404	F-box domain containing protein [Brugia malayi]	53036	R.QNALLIQPK.A	√	√	√
gi|170593721	CRAL/TRIO domain containing protein [Brugia malayi]	44167	K.MPGPTVVPIR.E + Oxidation (M)	√	√	√
gi|1707615	ATPase subunit 8 [Euhadra herklotsi]	1009	-.MPQLSPHSL.- + Oxidation (M)	√	√	√
gi|189236109	PREDICTED: similar to nucleoporin 37 [Tribolium castaneum]	36446	K.LLIAEKIGLIR.F	√	√	√
gi|193624646	PREDICTED: similar to muscle myosin heavy chain isoform 1 [Acyrthosiphon pisum]	225555	K.DQVGQVNPPK.Y	√	√	√
gi|193652752	PREDICTED: similar to exosome complex exonuclease RRP44 [Acyrthosiphon pisum]	94487	M.DFLENLSR.Q	√	√	√
gi|195996053	hypothetical protein TRIADDRAFT_51865 [Trichoplax adhaerens]	147604	R.DVTETLSTDV.- K.STHDVAVKLLR.D	√	√	√
gi|195997589	hypothetical protein TRIADDRAFT_51823 [Trichoplax adhaerens]	138255	R.GKLIQLQPK.T	√	√	√
gi|196007242	hypothetical protein TRIADDRAFT_57718 [Trichoplax adhaerens]	58245	K.VRYYQTPVR.D	√	√	√
gi|196012718	hypothetical protein TRIADDRAFT_60295 [Trichoplax adhaerens]	78546	R.LLDTTTTDAK.E	√	√	√
gi|207268055	ATP synthase F0 subunit 8 [Acrida willemsei]	6635	K.TITDNKEMK.T	√	√	√
gi|221109570	PREDICTED: similar to Uncharacterized protein F54H12.3 [Hydra magnipapillata]	38467	K.IKSNPIQPK.F	√	√	√
gi|221116331	PREDICTED: similar to dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 [Hydra magnipapillata]	67527	K.AQINVALKIIR.N	√	√	√

Appendix Table B12 (Continued)

Accession number	**Protein hits**	**Mw**	**Protein Sequence**	**Tick's gut protein**	**Bm95**	**Bm86**
gi|221131493	PREDICTED: similar to predicted protein, partial [Hydra magnipapillata]	40519	K.EGGPPKSKPK.K	√	√	√
gi|241161609	glycoprotein 6-alpha-L-fucosyltransferase, putative [Ixodes scapularis]	56983	-.TLTHRVR.L	√	√	√
gi|241561644	synembryn, putative [Ixodes scapularis]	14334	K.LARGGVVQPAR.V	√	√	√
gi|241594096	hypothetical protein IscW_ISCW009508 [Ixodes scapularis]	9092	-.MECLRSVLTIAGK.A	√	√	√
gi|241694276	conserved hypothetical protein [Ixodes scapularis]	34468	R.GGYLVKDLK.S	√	√	√
gi|241999218	hypothetical protein IscW_ISCW004240 [Ixodes scapularis]	34876	R.RGTDNAAVFK.F	√	√	√
gi|242005875	hypothetical protein Phum_PHUM086190 [Pediculus humanus corporis]	52382	K.NGGGDDDDDDDEGNGFGRGGGGGR.G	√	√	√
gi|242007156	mitochondrial 50S ribosomal protein L27, putative [Pediculus humanus corporis]	16163	-.MAALLLQLNSCSR.K	√	√	√
gi|242008059	calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase, putative [Pediculus humanus corporis]	121129	K.GGGGGGGGGGGGDK.K	√	√	√
gi|242013855	conserved hypothetical protein [Pediculus humanus corporis]	376680	K.CENMLAMKDETELNLNNR.I + Oxidation (M)	√	√	√
gi|242014857	hypothetical protein Phum_PHUM363220 [Pediculus humanus corporis]	66947	K.ILKNLAGKPK.I	√	√	√
gi|242021826	Angiotensin-converting enzyme precursor, putative [Pediculus humanus corporis]	81610	K.NYILLSNR.A	√	√	√
gi|242023447	conserved hypothetical protein [Pediculus humanus corporis]	48829	K.DFIENSLR.K	√	√	√
gi|242024421	micronuclear linker histone polyprotein subunit, putative [Pediculus humanus corporis]	75672	K.TLTFEEIRK.K	√	√	√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|256052100	Epa1p-like protein [Schistosoma mansoni]	65748	R.TITHSPVK.S	√	√	√
gi|256078034	initiation factor 5a [Schistosoma mansoni]	17855	K.DYQLVDIK.D	√	√	√
gi|256090402	gem-associated protein 5 (gemin5) [Schistosoma mansoni]	150695	K.SCEYIKPK.E	√	√	√
gi|268322308	alpha 1 type IV collagen [Haliotis tuberculata]	174650	R.GAPGLSGLKGPK.G	√	√	√
gi|268558454	Hypothetical protein CBG18883 [Caenorhabditis briggsae]	302891	R.CEIFNDVEKVETACIVER.I	√	√	√
gi|268570370	C. briggsae CBR-PAX-1 protein [Caenorhabditis briggsae]	24881	K.RADPGIFAWEIR.D	√	√	√
gi|268572719	Hypothetical protein CBG13254 [Caenorhabditis briggsae]	55921	K.NLMEITQK.F + Oxidation (M)	√	√	√
gi|268578497	Hypothetical protein CBG17235 [Caenorhabditis briggsae]	78492	R.SPELLLQPK.T	√	√	√
gi|268580415	C. briggsae CBR-PPK-3 protein [Caenorhabditis briggsae]	170200	R.EVDGTFMKVR.E	√	√	√
gi|268581039	Hypothetical protein CBG22754 [Caenorhabditis briggsae]	23318	K.ENQKPIKPK.T	√	√	√
gi|268582125	Hypothetical protein CBG10659 [Caenorhabditis briggsae]	47623	R.LLVASQPEVLI.-	√	√	√
gi|270010424	hypothetical protein TcasGA2_TC009817 [Tribolium castaneum]	376559	K.LMQLMVMK.H + 2 Oxidation (M)	√	√	√
gi|3608420	cyclin A [Dreissena polymorpha]	47955	R.VQPSRAAKPK.V	√	√	√
gi|37703935	elongation factor-2 [Eurytemora affinis]	79396	K.LTTXXGKXVK.D	√	√	√
gi|4191598	sarco(endo)plasmic reticulum-type calcium ATPase [Heliothis virescens]	110364	K.MNPFNVPK.T	√	√	√
gi|4218957	cytoplasmic polyadenylation element-binding protein [Spisula solidissima]	74011	R.NTKGHTNLALL.-	√	√	√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|56757477	SJCHGC00905 protein [Schistosoma japonicum]	40358	K.ELCKICWTEGLTLMLAWK.T + Oxidation (M)	√	√	√
gi|66535287	PREDICTED: similar to auxillin CG1107-PB, isoform B [Apis mellifera]	129521	R.LTTSMILER.L + Oxidation (M)	√	√	√
gi|72157528	PREDICTED: similar to conserved hypothetical protein [Strongylocentrotus purpuratus]	101408	K.KATEYGSTPK.W	√	√	√
gi|91081233	PREDICTED: similar to hormone-sensitive lipase [Tribolium castaneum]	84776	K.YKSKPPAR.G	√	√	√
gi|91093090	PREDICTED: similar to AGAP005527-PA [Tribolium castaneum]	165126	R.ILLDQRVGAPR.Y	√	√	√
gi|110751035	PREDICTED: similar to M-phase phosphoprotein 1 [Apis mellifera]	138300	K.LMVKYDDIK.I	√	√	
gi|115739527	PREDICTED: hypothetical protein [Strongylocentrotus purpuratus]	113994	R.MPPHLMMDK.G	√	√	
gi|115896473	PREDICTED: similar to MGC83212 protein [Strongylocentrotus purpuratus]	49626	K.LMKQDEYR.S	√	√	
gi|119370319	T-box protein TbxE [Mnemiopsis leidyi]	27268	K.NSSAHPSCLK.E	√	√	
gi|121256	RecName: Full=Globin CTT-IIIA	16144	K.ALLNQLGTSHK.A	√	√	
gi|156379770	predicted protein [Nematostella vectensis]	39226	K.DEVKHIQGGAK.A	√	√	
gi|156380891	predicted protein [Nematostella vectensis]	31522	K.IHMPPGSK.V + Oxidation (M)	√	√	
gi|156384835	predicted protein [Nematostella vectensis]	18421	K.SGAKQSPFFR.K	√	√	
gi|156393510	predicted protein [Nematostella vectensis]	14503	R.NKTQDIIHR.T	√	√	
gi|156408383	predicted protein [Nematostella vectensis]	56096	R.HSQVNSKPER.G	√	√	

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|156540708	PREDICTED: similar to tyrosine recombinase [Nasonia vitripennis]	62808	K.NQTKLLLSLYK.E	√	√	
gi|156541190	PREDICTED: similar to signal recognition particle receptor alpha subunit [Nasonia vitripennis]	69406	K.KQNKPIEVPK.V	√	√	
gi|157120630	hypothetical protein AaeL_AAEL009087 [Aedes aegypti]	31539	R.YHMVIHGPK.K	√	√	
gi|157123807	golgi-specific brefeldin a-resistance factor [Aedes aegypti]	214572	K.DIVLLLFMR.L + Oxidation (M)	√	√	
gi|157125153	hypothetical protein AaeL_AAEL010109 [Aedes aegypti]	20735	K.TISTVMNT.- + Oxidation (M)	√	√	
gi|157125808	membrane traffic protein [Aedes aegypti]	51628	R.MAALVCIPQTTSK.R	√	√	
gi|157134125	hypothetical protein AaeL_AAEL003092 [Aedes aegypti]	174099	R.FDLIEDNK.V	√	√	
gi|158284931	AGAP002202-PA [Anopheles gambiae str. PEST]	26868	R.LTTDIPYEK.M	√	√	
gi|170051303	aminopeptidase N [Culex quinquefasciatus]	104069	R.AASVLNMFR.Q	√	√	
gi|170583347	hypothetical protein Bm1_25390 [Brugia malayi]	22336	K.TPVTPKQIVLK.N	√	√	
gi|170586382	DEAH [Brugia malayi]	77562	K.KTVLNMFR.D	√	√	
gi|170592699	ABC transporter family protein [Brugia malayi]	253101	R.DNYLIDLK.Y	√	√	
gi|170596587	3'-5' exonuclease family protein [Brugia malayi]	81434	R.AGTVESFAAKR.A	√	√	
gi|189233623	PREDICTED: similar to fibroblast growth factor receptor [Tribolium castaneum]	109725	K.MIDIETVR.A + Oxidation (M)	√	√	
gi|193668032	PREDICTED: similar to AGAP007175-PA [Acyrthosiphon pisum]	62018	K.LKGPK.H / K.EDFDSEDDK.N	√	√	

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|196005577	hypothetical protein TRIADDRAFT_56895 [Trichoplax adhaerens]	72241	K.NDVLSSVMK.A + Oxidation (M)	√	√	
gi|221130433	PREDICTED: similar to thioredoxin domain containing 11 [Hydra magnipapillata]	61790	R.LINRVNKPK.I	√	√	
gi|240270422	serpin 10 plasmodium-related inhibitory serine protease inhibitor [Anopheles gambiae]	24471	K.QTVIDNYR.R	√	√	
gi|241155753	excitatory amino acid transporter, putative [Ixodes scapularis]	102695	K.DIVIRHVR.D	√	√	
gi|242003806	conserved hypothetical protein [Pediculus humanus corporis]	86129	R.EKYCPLVTNGEGMELLEELAK.A + Oxidation (M)	√	√	
gi|242005496	conserved hypothetical protein [Pediculus humanus corporis]	117349	K.LLPKSIEPVK.M	√	√	
gi|242007326	conserved hypothetical protein [Pediculus humanus corporis]	130065	K.DLVHEMVLK.K + Oxidation (M)	√	√	
gi|242010126	Nuclear RNA export factor, putative [Pediculus humanus corporis]	71855	R.IVYLEDNK.I	√	√	
gi|254933957	carbamoylphosphate synthetase [Euclemensia bassettella]	109474	K.WLESYTHSSITFEILKGAK.Q	√	√	
gi|256070183	hypothetical protein [Schistosoma mansoni]	45798	K.KPLTRNKPVK.T	√	√	
gi|256073891	hypothetical protein [Schistosoma mansoni]	61705	K.GNLDTSVSQTSISTASRSPSSK.L	√	√	
gi|256075113	brefeldin A-inhibited guanine nucleotide-exchange protein [Schistosoma mansoni]	230732	R.LSKSHIGHFLGENEPYNLR.V	√	√	
gi|268536920	Hypothetical protein CBG05472 [Caenorhabditis briggsae]	37769	K.GGTGFGVMDLLNQTVSQSIFGPR.R	√	√	
gi|268552739	Hypothetical protein CBG17702 [Caenorhabditis briggsae]	55277	R.DSAITGEQINEDK.I	√	√	
gi|268554292	C. briggsae CBR-MYO-5 protein [Caenorhabditis briggsae]	219524	R.AAATETFRLR.N R.KVEGDLQHLQSEVEEALSDAK.V	√	√	

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|268556740	Hypothetical protein CBG08656 [Caenorhabditis briggsae]	46743	-.DQIKMASEWGYNQEDIIAALK.E + Oxidation (M)	√	√	
gi|29840983	hypothetical protein [Schistosoma japonicum]	36215	K.TLTHGKPK.C	√	√	
gi|3660698	period [Cydia pomonella]	39618	R.ILNEVPTRPAELVKQQMTK.R + Oxidation (M)	√	√	
gi|3892088	prophenoloxidase [Anopheles gambiae]	78684	K.FTVTLNAGANTIVR.R	√	√	
gi|58378650	AGAP006821-PA [Anopheles gambiae str. PEST]	42046	-.MAALTKGVFIVAAK.R	√	√	
gi|66507736	PREDICTED: similar to Elongin B CG4204-PA [Apis mellifera]	13138	K.DNVLMSDSK.F	√	√	
gi|66509122	PREDICTED: similar to CG11594-PA, isoform A isoform 1 [Apis mellifera]	60540	K.AMGGSATMVKPR.S + 2 Oxidation (M)	√	√	
gi|76155338	SJCHGC05221 protein [Schistosoma japonicum]	37634	-.PFQDEYKR.F	√	√	
gi|115647211	PREDICTED: similar to MGC80611 protein [Strongylocentrotus purpuratus]	23945	K.ELTAGLSQYNK.S	√		√
gi|115712231	PREDICTED: similar to LOC495952 protein, partial [Strongylocentrotus purpuratus]	41446	R.LDGKGQGGSK.G	√		√
gi|118783458	AGAP004126-PA [Anopheles gambiae str. PEST]	24643	K.SSISLGDSFELMK.T + Oxidation (M)	√		√
gi|15425683	Kettin [Procambarus clarkii]	546739	R.IDWFRDGQPIQMGSR.F + Oxidation (M)	√		√
gi|156321438	hypothetical protein NEMVEDRAFT_v1g225319 [Nematostella vectensis]	26748	K.LVMRHAGLLR.K + Oxidation (M)	√		√
gi|156328522	hypothetical protein NEMVEDRAFT_v1g224666 [Nematostella vectensis]	51557	K.ARECLEK.L	√		√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|156340946	hypothetical protein NEMVEDRAFT_v1g147631 [Nematostella vectensis]	41345	K.LLVIVSRPAR.L	√		√
gi|156401368	predicted protein [Nematostella vectensis]	55046	K.DISQTLANFK.K	√		√
gi|156543280	PREDICTED: hypothetical protein [Nasonia vitripennis]	129511	K.HSDSLLKILR.N	√		√
gi|157106155	myosin [Aedes aegypti]	180581	K.NLSSDITR.S	√		√
gi|157106621	DNA polymerase eta [Aedes aegypti]	95165	R.KRPTSGDK.S	√		√
gi|158294659	scavenger receptor class B (AGAP005725-PA) [Anopheles gambiae str. PEST]	60310	K.TYFKIQPK.L	√		√
gi|158297905	AGAP004765-PA [Anopheles gambiae str. PEST]	82488	K.MLKGEYTLK.A	√		√
gi|170046628	U3 small nucleolar ribonucleoprotein protein MPP10 [Culex quinquefasciatus]	75364	K.LLEKGIKPK.G	√		√
gi|170059237	conserved hypothetical protein [Culex quinquefasciatus]	117287	K.TLKTIGGYVK.N	√		√
gi|18307516	putative venom metalloprotease [Pimpla hypochondriaca]	61759	R.CTHSRPVGVPK.G	√		√
gi|189237783	PREDICTED: similar to 2-phosphodiesterase [Tribolium castaneum]	65715	R.DKSTWVQVANTFR.Y	√		√
gi|196006884	hypothetical protein TRIADDRAFT_7238 [Trichoplax adhaerens]	15711	K.TAAKGSPFFR.K	√		√
gi|20799320	fasciclin-like protein [Aplysia californica]	87461	R.INVIDDVMVPPK.G + Oxidation (M)	√		√
gi|211938970	Chain A, A Non-Cytotoxic Dsred Variant For Whole-Cell Labeling	24965	K.SIYMAKKPVK.L + Oxidation (M)	√		√
gi|221106965	PREDICTED: similar to CG31809 CG31809-PB, partial [Hydra magnipapillata]	28677	K.LNVLLICKNTDK.L	√		√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|221122797	PREDICTED: similar to Ift46 protein [Hydra magnipapillata]	60790	R.NNDGFLYNPK.D	√		√
gi|242017225	DNA polymerase epsilon, catalytic subunit A, putative [Pediculus humanus corporis]	261671	K.LIDLKNNPVR.N	√		√
gi|268531878	C. briggsae CBR-TRR-1 protein [Caenorhabditis briggsae]	474950	R.DLEMIGYTAK.M + Oxidation (M)	√		√
gi|268577861	Hypothetical protein CBG02177 [Caenorhabditis briggsae]	76713	R.LPNPLLSVAR.E	√		√
gi|268578537	Hypothetical protein CBG13997 [Caenorhabditis briggsae]	25220	K.LLKIEQLLL.-	√		√
gi|270013886	hypothetical protein TcasGA2_TC012552 [Tribolium castaneum]	92073	R.MSSSGVSEMQFSMRSR.T + Oxidation (M)	√		√
gi|289741131	OTU-like cysteine protease [Glossina morsitans morsitans]	37743	K.IDNEGSEMMR.H	√		√
gi|29840923	similar to NM_031954 MSTP028 protein in Homo sapiens [Schistosoma japonicum]	39450	R.NIECLEK.Y	√		√
gi|3023865	RecName: Full=Guanine nucleotide-binding protein G(q) subunit alpha; AltName: Full=Guanine nucleotide-binding protein alpha-q	41553	R.INCEIEK.E	√		√
gi|3183022	RecName: Full=50 kDa hatching enzyme; Short=HEZ; Short=HE; AltName: Full=Envelysin; AltName: Full=Sea-urchin-hatching proteinase; Contains: RecName: Full=38 kDa hatching enzyme; Contains: RecName: Full=32 kDa hatching enzyme non-specific; Contains:	66485	R.DDIAGITSLYGR.N	√		√
gi|3426184	SEVENLESS [Anopheles sp.]	99503	R.CQNTSEDTQIIAPFPATCSK.V	√		√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|56756192	SJCHGC09241 protein [Schistosoma japonicum]	49876	K.VENGPRKPK.E	√		√
gi|60691514	SJCHGC05193 protein [Schistosoma japonicum]	9688	R.DKQPNGPLR.S	√		√
gi|72167364	PREDICTED: similar to aquaporin 4, partial [Strongylocentrotus purpuratus]	33778	K.DDLGSADFWR.A	√		√
gi|78190747	succinate dehydrogenase iron-sulfur protein [Aphrocallistes vastus]	11439	R.INTDSSKVSK.I	√		√
gi|91079981	PREDICTED: similar to AGAP005961-PA [Tribolium castaneum]	91409	K.GTGKQLHDAVR.V	√		√
gi|91081321	PREDICTED: similar to CG5198 CG5198-PA [Tribolium castaneum]	36861	K.MLNYMKPK.E	√		√
gi|110734693	elongation factor-1 gamma [Uloborus diversus]	48763	K.LGGEKPAKPK.E		√	√
gi|115634798	PREDICTED: similar to ENSANGP00000007239 [Strongylocentrotus purpuratus]	305747	R.EKHDSSPGILR.Q		√	√
gi|115749236	PREDICTED: similar to BIG3, partial [Strongylocentrotus purpuratus]	169014	K.MEAAHIKGIIR.A		√	√
gi|118781575	AGAP010405-PA [Anopheles gambiae str. PEST]	17503	K.TAQSMMRPK.S + 2 Oxidation (M)		√	√
gi|156362206	predicted protein [Nematostella vectensis]	34377	R.TPGSEGHRIVK.T		√	√
gi|156392182	predicted protein [Nematostella vectensis]	57541	R.GSAPPPPPAR.M		√	√
gi|156546758	PREDICTED: similar to ATP-binding cassette sub-family A member 3, putative [Nasonia vitripennis]	187844	R.MPNEFYDVACK.S		√	√
gi|157713480	nicotinic acetylcholine receptor beta subunit [Haemonchus contortus]	55417	R.TLGAQEYSVR.V		√	√

Appendix Table B12 (Continued)

Accession number	Protein hits	Mw	Protein Sequence	Tick's gut protein	Bm95	Bm86
gi|158291121	AGAP002345-PA [Anopheles gambiae str. PEST]	87465	R.QCGAIFPHHENMSLLQQQK.L + Oxidation (M)		√	√
gi|158293448	AGAP008690-PA [Anopheles gambiae str. PEST]	63872	K.VLLLNGAFQDGRK.L		√	√
gi|158301484	AGAP001899-PA [Anopheles gambiae str. PEST]	266844	R.NMLDKDIK.S + Oxidation (M)		√	√
gi|193603470	PREDICTED: similar to molting fluid carboxypeptidase A [Acyrthosiphon pisum]	58753	K.TADLVLPPSVAR.D		√	√
gi|193624948	PREDICTED: similar to gag-like protein [Acyrthosiphon pisum]	48472	R.STLVKEEASFK.R		√	√
gi|195999698	predicted protein [Trichoplax adhaerens]	58417	R.NVTEYLLAR.L		√	√
gi|196015769	hypothetical protein TRIADDRAFT_33038 [Trichoplax adhaerens]	479542	K.LNFENDLK.I		√	√
gi|221122039	PREDICTED: similar to TNF-receptor-associated factor 1 [Hydra magnipapillata]	44059	R.EPVLTLCGHR.L		√	√
gi|241162965	hypothetical protein IscW_ISCW016627 [Ixodes scapularis]	9338	R.ERPFGAGR.E		√	√
gi|256073417	hypothetical protein [Schistosoma mansoni]	51173	R.LVQRLDSMISLR.R		√	√
gi|268531518	Hypothetical protein CBG02606 [Caenorhabditis briggsae]	58116	R.YHQKPLAPK.V		√	√
gi|91076832	PREDICTED: similar to GA20540-PA [Tribolium castaneum]	145934	R.VQSEFDQVK.L		√	√
gi|91090029	PREDICTED: similar to glycerol-3-phosphate acyltransferase [Tribolium castaneum]	98975	R.NFLKDDLK.E		√	√

Total protein = 187 identification proteins.

Appendix C

Liquid – Chromatography Mass Spectrometry instument

Appendix Figure C1 The HPLC instrument, Ultimate 3000 LC system (Dionex, USA)

Appendix Figure C2 The ESI – Ion Trap MS instrument, HCT Ultra PTM Discovery System (Bruker, Germany)

Appendix Figure C3 LCMS system, LCMS coupled to ESI – Ion Trap MS.

CIRRICULUM VITAE

NAME : Mr. Sinsamut Saengow

BIRTH DATE : November 9, 1979

BIRTH PLACE : Bangkok, Thailand

EDUCATION :

YEAR	**INSTITUTE**	**DEGREE**
2004	Kasetsart University	D.V.M.

WORK PLACE **:** Faculty of Veterinary Medicine, Kasetsart University

PUBLICATION

Jittapalapong S., A. Sangvaranond, T. Inpankaew, N. Pinyopanuwat, W. Chimnoi, C. Kengradomkij, S. Saengow and S. Wongnakphet. 2007. Ectoparasites of Stray Cats in Bangkok Metropolitan Areas, Thailand. *In* **The 46th Kasetsart University Annual Conference** (29 Jan-1 Feb, 2008).

______., N. Pinyopanuwat, W. Chimnoi, B. Bimsupan, S. Saengow, P. Simking and K. Kaewmongkol. 2005. Prevalence of heartworm infection of stray dogs and cats in Bangkok metropolitan areas. **Kasesart.J.(Nat.Sci.)** 39:30 – 34

______, T. Inpankaew, A. Sangwaranond, C. Phasuk, N. Pinyopanuwat, W. Chimnoi, C. Kengradomkij, S. Saengow, P. Pumhom, Pipat Arunwipat, T. Anakewit and I. D. Robertson. 2008. Seroprevalence of *Neospora caninum* Infections of Dairy Cows in the North-east of Thailand. **Kasesart.J.(Nat.Sci.)** 42:61 – 66