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# Investigating the Proteome of Tardigrades: Towards a Better Understanding of Molecular Mechanisms during Anhydrobiosis

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*For my parents*



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## Publications

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“Modulation of oxidative phosphorylation machinery signifies a prime mode of anti-ageing mechanism of calorie restriction in male rat liver mitochondria”

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Transcriptome analyzed at different levels in *Hypsibius dujardini* and *Milnesium tardigradum*: Specific adaptations, motifs and clusters as well as general protective pathways, in preparation

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## Presentations at national and international scientific conferences

### Oral presentations

**“Functional analysis of dynamic processes in anhydrobiotic tardigrade *Milnesium tardigradum*”**

Elham Schokraie, Ralph. O. Schill, and Martina Schnölzer

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**“Cheating Death, What we can learn from the nature!”**

Elham Schokraie, Ralph. O. Schill, and Martina Schnölzer

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**“Towards a two-dimensional protein reference map of *Milnesium tardigradum*”**

Elham Schokraie, Uwe Warnken, Agnes Hotz-Wagenblatt, Brahim Mali, Marcus Frohme, Frank Förster, Thomas Dandekar, Steffen Hengherr, Ralph. O. Schill, and Martina Schnölzer

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Elham Schokraie, Uwe Warnken, Agnes Hotz-Wagenblatt, Brahim Mali, , Marcus Frohme, Frank Förster, Thomas Dandekar, Steffen Hengherr, Ralph. O. Schill, and Martina Schnölzer

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**“Comparative proteomic analysis of tardigrades in active *versus* anhydrobiotic state using DIGE technology”**

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**“Investigating the proteome of *Milnesium tardigradum*: towards a better understanding of molecular mechanisms by anhydrobiotic organisms”**

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## Poster presentations

### **“Functional analysis of dynamic processes in cryptobiotic tardigrades”**

Elham Schokraie, Ralph. O. Schill, Martina Schnölzer

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### **“Proteomic analysis of cryptobiotic tardigrade *Milnesium tardigradum* and differential analysis of tardigrades in active and tun state“**

Elham Schokraie, Tore Kempf, Uwe Warnken, Ralph. O. Schill, Martina Schnölzer

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### **“Functional analysis of dynamic processes in anhydrobiotic tardigrade *Milnesium tardigradum*”**

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### **“Comparative proteome analysis of desiccation stress response in *Milnesium tardigradum* using DIGE technology”**

Elham Schokraie, Ralph. O. Schill and Martina Schnölzer

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### **“Phosphoprotein profiling of tardigrades in active and anhydrobiotic state”**

Elham Schokraie, Uwe Warnken and Martina Schnölzer

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### **“Investigating the proteome of *Milnesium tardigradum*”**

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### **“De novo sequence protein analysis of *Milnesium tardigradum*”**

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## Abstract

Tardigrades have fascinated researchers for more than 300 years because of their amazing capability to undergo anhydrobiosis. In extreme states of dehydration, anhydrobiotic tardigrades undergo a metabolic dormancy, in which metabolism decreases to a non-measurable level and life comes to a reversible standstill until activity is resumed under more favourable conditions. In the anhydrobiotic (tun) state, tardigrades are extraordinary tolerant to physical extremes including high and subzero temperatures, high pressure, and extreme levels of ionizing radiation. Possessing the ability to enter this ametabolic state at any developmental stage, tardigrades are capable of surviving for a very long time and extend their lifespan significantly. Anhydrobiosis seems to be the result of dynamic processes and appears to be mediated by protective systems that prevent lethal damage. However, the survival mechanisms of tardigrades are still poorly understood. This is mainly caused by notable absence of detailed analysis concerning the proteome and genome of these organisms.

FUNCRYPTA (Functional Analysis of Dynamic Processes in Cryptobiotic Tardigrades) project consisting of four research groups has been established to fill this gap by performing a broad range of investigations and analyses. As Funcrypta's cooperation partner specialized in proteomics field we started with establishing optimal protocols for extraction of proteins from tardigrades, performing high resolution gel electrophoresis and high throughput protein identification and quantification. Since the presence of a comprehensive protein database is a prerequisite for protein identification, a *M. tardigradum* sequencing project has been initiated in parallel to our proteomic study by our genomic cooperation partner. The first tardigrade protein database translated from expressed sequence tags (ESTs), that have been generated by Sanger sequencing contained 3318 sequences. This protein database allowed us to develop the first proteome map of tardigrades utilizing 2D gel electrophoresis. The second protein database based on 454 sequencing with a high number of 24679 protein sequences provided us the basis for protein identification and quantification in a large scale. This resulted for the first time in a broad characterization of proteins expressed in tardigrades. More than 3000 unique proteins of *M. tardigradum* in three different states (early embryonic state and adults in active and anhydrobiotic states) have been identified with high sequence coverage using 1D electrophoresis in combination with high sensitive nanoLC ESI-MS/MS on a LTQ-Orbitrap mass spectrometer. Among the broad range of identified protein families, proteins known to be associated with desiccation tolerance were identified. This includes proteins with antioxidant activity, chaperones in particular heat shock proteins, aquaporins and Late Embryogenesis Abundant (LEA) proteins. Furthermore the present study provides a semi-quantitative analysis of proteins expressed in early embryonic state

and adults in active and anhydrobiotic states using a label-free approach based on Exponentially Modified Protein Abundance Index (emPAI). This method allowed the classification of proteins present in one state in major and minor components and furthermore a quantitative analysis of differentially expressed proteins in each state. The semi-quantitative analysis delivered consequential results in comparing early embryonic state and adults, which will be of importance in the field of developmental biology. Using this approach we quantitatively analyzed the expressed heat shock proteins in active and tun states. The success of the analysis could be confirmed, by the published gene expression analysis of some heat shock proteins performed by our cooperation partner, which delivered similar results. The semi-quantitative analysis of active *versus* tun state demonstrated up-regulation of proteins in tun state that are mainly not annotated, since they are tardigrade specific and the homology search delivered no result. The functional analysis of these specific proteins in future investigations will be of major importance in regard to investigating anhydrobiosis.

Analyzing the proteins that are only identified in tun state, leads to the assumption that not only proteins such as chaperones play important roles in protection mechanisms during anhydrobiosis, but also further processes and mechanisms are associated such as phosphorylation and activation of intracellular signalling cascades. Therefore optimal protocols for analyzing phosphoproteins in tardigrades have been developed and first experiments in detecting phosphoproteins on 2D gels using fluorescent dye (ProQ Diamond) have been performed.

This comprehensive study from the first step of developing optimized protocol for protein extraction to the large scale protein identification and quantification builds the basis for future investigations in the field of anhydrobiotic organisms in regard to isolation and functional characterization of proteins, which are associated with protection mechanisms during anhydrobiosis. Understanding the desiccation-tolerance in anhydrobiotic tardigrades will probably enable us to develop new strategies for long-term stabilization and preservation of biological macromolecules in the future, which will be immensely important in medical field as well as in pharmaceutical industry.

## Zusammenfassung

Tardigraden haben seit mehr als 300 Jahren die Forscher durch ihre einzigartige Fähigkeit, Anhydrobiose einzugehen, fasziniert. Anhydrobiose wird durch extreme Trocknung ausgelöst. Dabei ändern Tardigraden ihre Körperform, ziehen sich zusammen und bilden ein Tönnchen, in dem keinerlei Metabolismus mehr nachweisbar ist. Im anhydrobiotischen (Tönnchen-) Stadium zeigen Tardigraden eine außergewöhnliche Toleranz gegenüber physikalischen Extremen wie hohem Druck, hohen oder niedrigen Temperaturen und ionisierender Strahlung. Durch die Fähigkeit in jeder Entwicklungsphase Anhydrobiose einzugehen, können Tardigraden ihre Lebensdauer deutlich verlängern. Die molekularen Schutz- und Reparatur-Mechanismen während der Anhydrobiose sind aufgrund fehlender fundamentalen Untersuchungen im genomischen und proteomischen Bereich noch unklar.

Die molekularen Prozesse während der Anhydrobiose und die involvierten Mechanismen können nur dann geklärt werden, wenn die Grundlagen erforscht sind und für weitergehende Experimente zur Verfügung stehen. Das FUNCRYPTA (Funktionelle Analyse Dynamischer Prozesse in Anhydrobiotischer Tardigraden) Projekt bestehend aus vier Forschungsgruppen hat sich zum Ziel gesetzt, diese Grundlagen zu erforschen. Als Kooperationspartner spezialisiert in Proteomik haben wir in der folgenden Studie optimierte Protokolle für Proteinextraktion, Gelelektrophorese mit hoher Auflösung und Protein-identifikation und -quantifizierung etabliert. Da eine der wichtigsten Voraussetzungen für die Proteome-Analyse die Existenz einer möglichst vollständigen Proteindatenbank ist, wurde zunächst von unseren Kooperationspartnern eine spezifische Proteindatenbank aus EST-Sequenzen, die durch Sanger-Sequenzierung erzeugt waren, entwickelt. Diese erste Proteindatenbank, die 3318 Proteinsequenzen enthielt, ermöglichte uns die Entwicklung der ersten Proteomkarte vom *M. tardigradum*. Die zweite Proteindatenbank, die auf 454 Sequenzierung basiert und 24679 Proteinsequenzen beinhaltet, lieferte eine gute Basis für umfangreiche Identifizierung und Quantifizierung der Proteine. Mehr als 3000 einzelne Proteine konnten durch 1D-Elektrophorese in Kombination mit hoch sensitiven massenspektrometrischen Methoden in drei verschiedenen Lebenszuständen der Tardigraden (Embryonen im Frühstadium, erwachsenen Tiere im Aktiv- und Tönnchen-Zustand) identifiziert werden. Diverse Proteinfamilien involviert in ein breites Spektrum biologischer Prozesse sind in den Ergebnissen erhalten. Es konnten vor allem einige Proteine, deren Assoziation mit Anhydrobiose bereits in anderen Organismen untersucht worden sind, in Tardigraden identifiziert werden. Dazu gehören Proteine mit Antioxidant-Aktivität, „Chaperone“-Proteine wie Hitzeschock-Proteine und weiterhin LEA (Late Embryogenesis Abundant) Proteine und Aquaporine.

Zusätzlich wurde in der folgenden Studie eine semi-quantitative Analyse der vorhandenen Proteine in drei verschiedenen Lebensstadien (Embryonen, erwachsenen Tiere in Aktiv- und Tönnchen-Zustand) durchgeführt. Der Vergleich und die Quantifizierung identifizierter Proteine durch eine label-freien Methode lieferte aufschlussreiche Ergebnisse über exprimierten Proteine im Embryonen-Frühstadium und Hinweise über mögliche Proteine und Mechanismen, die dem Prozess der Anhydrobiose assoziiert sind. Der Erfolg dieser Quantifizierungsmethode für ausgewählte Hitzeschock-Proteine konnte durch publizierte Gen-Expressionsdaten von unserem Kooperationspartner bestätigt werden. Die semi-quantitative Analyse der exprimierten Proteinen in Aktiv- versus Tönnchen-Zustand zeigte die Hochregulation vieler Proteine, die durch Homologie-Suche nicht annotiert werden konnten. Die funktionelle Analyse und Charakterisierung dieser Tardigraden spezifischen Proteine ist der nächste wichtige Schritt in der Tardigraden-Forschung.

Die funktionelle Analyse der Proteine, die nur im Tönnchen-Zustand identifiziert wurden, führte zur Annahme, dass Prozesse wie Phosphorylierung und Aktivierung der intrazellulären Kaskaden eine wichtige Rolle während der Anhydrobiose spielen können. Aus diesem Grund wurde in ersten Experimenten die Phosphorylierung als posttranslationale Modifikation untersucht. Dazu wurden Protokolle zur Detektion der Phosphoproteine in 2D-Gelen durch den Fluoreszenz-Farbstoff ProQ Diamond optimiert.

Die proteomischen Ergebnisse zusammen mit erzielten genetischen und bioinformatischen Untersuchungen und Auswertungen stellen nicht nur die fundamentalen Grundlagen der Tardigraden-Forschung dar, sondern liefern viele neue Erkenntnisse auf dem Weg zur Aufklärung der involvierten Schutz-Mechanismen bei der Anhydrobiose. Dies kann in Zukunft zur Entwicklung neuer Methoden und Strategien für die Konservierung und Stabilisierung biologischer Materialien führen, die einen wichtigen Beitrag im Bereich Medizin und Pharma liefern.



## Abbreviations

µg	microgramm
µL	microliter
µm	micrometer
2D	two-dimensional
AS	active state
BVA	biological variation analysis
Da	dalton
ddH <sub>2</sub> O	double distilled H <sub>2</sub> O
DIA	differential in-gel analysis
DIGE	fluorescence difference gel electrophoresis
EES	early embryonic state
emPAI	exponentially modified protein abundance index
ESI	electrospray ionization
EST	expressed sequence tag
FUNCRYPTA	Functional Analysis of Dynamic Processes in Cryptobiotic Tardigrades
ICAT	isotope-coded affinity tag
ICPL	isotope coded protein label
IEF	isoelectric focussing
iTRAQ	isobaric tags for relative and absolute quantification
kDa	kilodalton
kV	kilovolt
log	logarithmus
LTQ	linear trap quadrupole
M	molar
m/z	mass to charge ratio
MALDI	matrix assisted laser desorption/ionization
mg	milligramm
min	minute
mL	milliliter
mM	millimolar
MS	mass spectrometry
MW	molecular weight
MS/MS	tandem mass spectrometry
N	nitrogen
ng	nanogramm
nm	nanometer
O	oxygen
p.a.	pro analysis
PAGE	polyacrylamide gel electrophoresis
PAI	protein abundance index
pI	isoelectric point
pmol	picomole
ppm	parts per million
PTM	posttranslational modification
Q	quadrupole
SDS	sodium dodecyl sulfate

SEM	Scanning Electron Microscope
SILAC	stable isotope labeling by amino acids in cell culture
TMT	tandem mass tags
TS	tun state
V	Volt
v/v	volume per volume
w/v	weight per volume

# Table of contents

<b>Publications</b>	<b>VII</b>
<b>Presentations at national and international scientific conferences</b>	<b>IX</b>
<b>Acknowledgements</b>	<b>XI</b>
<b>Abstract</b>	<b>XIII</b>
<b>Zusammenfassung</b>	<b>XV</b>
<b>Abbreviations</b>	<b>XVII</b>
<b>Table of contents</b>	<b>XIX</b>
<b>1 Introduction</b>	<b>1</b>
1.1 <i>Milnesium tardigradum</i> , a model organism for investigating anhydrobiosis	1
1.2 <b>FUNCRIPTA</b> project and bioinspired products	2
1.3 Involvement of disaccharides and proteins in anhydrobiosis	3
1.4 Analyzing the proteome of tardigrades using high throughput proteomics technologies	5
1.4.1 Proteome profiling	5
1.4.2 Quantitative analysis of tardigrades in three different states	6
<b>2 Materials and methods</b>	<b>8</b>
2.1 Materials	8
2.2 Methods	9
2.2.1 From tardigrade sampling to proteome analysis	9
2.2.2 Sampling of tardigrades in early embryonic state	9
2.2.3 Sample preparation for phosphoprotein analysis	10
2.2.4 Two-dimensional fluorescence difference gel electrophoresis (2D DIGE)	10
2.2.5 Phosphoprotein detection	11
<b>3 Results and discussion</b>	<b>13</b>
3.1 Comprehensive proteome profiling of <i>M. tardigradum</i>	13
3.1.1 Developing a 2D proteome map	13
3.1.2 Proteome analysis using 1D gel electrophoresis	18
3.1.3 Comparative analysis of identified proteins in different states	25
3.2 Quantitative analysis of proteins in tardigrades in different states	30
3.2.1 Two-dimensional fluorescence difference gel electrophoresis (2D DIGE)	30
3.2.2 Semi-quantitative analysis using a label-free approach based on Exponentially Modified Protein Abundance Index (emPAI)	33
3.3 Detection of phosphoproteins on 2D gels	46
<b>4 Conclusion</b>	<b>48</b>
<b>5 References</b>	<b>50</b>
<b>6 Manuscripts</b>	<b>54</b>
6.1 Proteomic analysis of tardigrades: towards a better understanding of molecular mechanisms by anhydrobiotic organisms	54

6.2	<i>Investigating heat shock proteins of tardigrades in active versus anhydrobiotic state using shotgun proteomics</i>	92
7	<i>Supplementary</i>	127
7.1	<i>Supplementary I</i>	127
7.2	<i>Supplementary II</i>	160
7.3	<i>Supplementary III</i>	229
8	<i>Curriculum Vitae</i>	233

# 1 Introduction

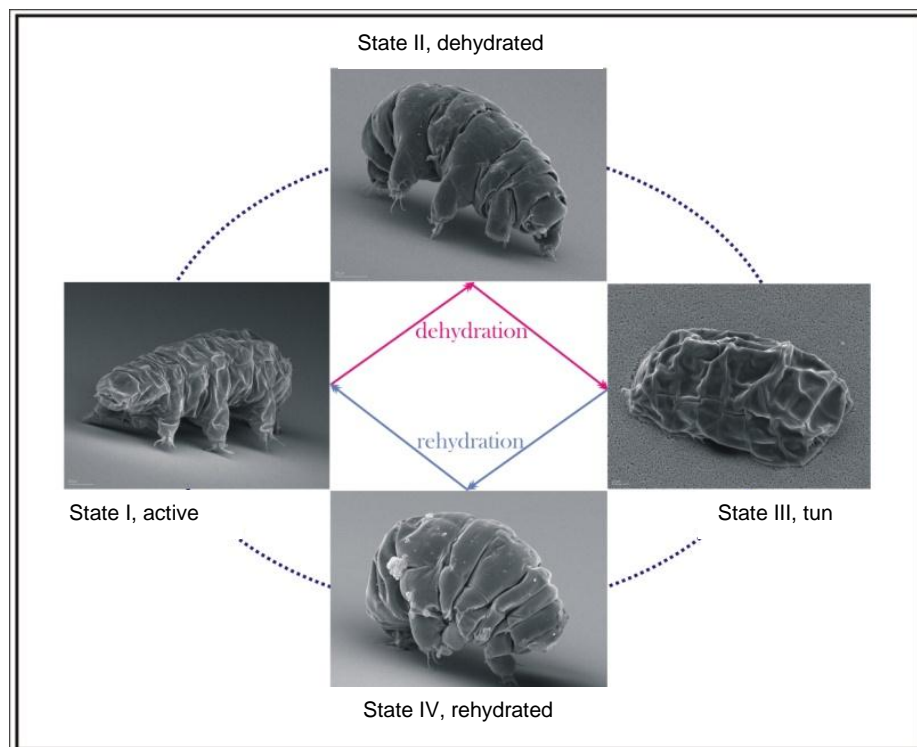
## 1.1 *Milnesium tardigradum*, a model organism for investigating anhydrobiosis

Many organisms are exposed to unfavourable, stressful environmental conditions, either permanently or for just certain periods of their lives. To survive these extreme conditions, they possess different mechanisms. One of amazing adaptation is anhydrobiosis (from the Greek for "life without water"), which has puzzled scientists for more than 300 years. For the first time the Dutch microscopist Anton van Leeuwenhoek (1702) gave a formal description of this phenomenon. He reported the revival of "animalcules" from rehydrated moss samples. In extreme states of dehydration, anhydrobiotic invertebrates undergo a metabolic dormancy, in which metabolism decreases to a non-measurable level and life comes to a reversible standstill until activity is resumed under more favourable conditions [1]. One of the best known anhydrobiotic organisms are tardigrades. Tardigrades are small invertebrates with a body length of 0.1-1.0 mm. *Milnesium tardigradum* Doyère (1840) belongs to the species of carnivorous tardigrades and is analyzed already by different aspects of its life history [2, 3]. Almost all reported tardigrade cultures consist only of females and the reproduction occurs by parthenogenesis. Up to seven periods of moult could be detected in one life cycle. The species grow into mature adults after the second moulting [2, 3] and the first period of egg laying accompanies the third moulting process. The eggs are laid in the space between the old and new cuticles and females remain within the old cuticle, along with the developing embryos, for several hours after egg laying [3].

Tardigrades have been in focus in the last decades because of their amazing capability to survive extreme environmental conditions. Anhydrobiosis is a form of cryptobiosis, which appears by water lost and desiccation. There are two known strategies to cope with water deficiency: "desiccation-avoidance strategy" and "desiccation-tolerance strategy" [4]. The term "desiccation-avoidance strategy" describes physiological and morphological adaptations to reduce water loss. To prevent the over-dehydration the African lungfish for example build a waterproof cocoon [4]. Tardigrades remain in their active form when they are surrounded by at least a film of water. By losing most of their free and bound water (>95%) anhydrobiosis occurs [5]. Tardigrades begin to contract their bodies and change their body structure into a so-called tun state (Figure 1). In the dry state these organisms are highly resistant to environmental challenges and they may remain dormant for a long period of time. "Desiccation-tolerance strategy" is used for withstanding the dehydrated state. The best example for this strategy is the anhydrobiosis, when the metabolic activity is reversibly at a standstill.

Based on their amazing capability to undergo anhydrobiosis, tardigrades colonise a diversity of extreme habitats [6], and they are able to tolerate harsh environmental conditions in any

developmental stage [7]. Possessing the ability to enter anhydrobiosis at any stage of life cycle, tardigrades can extend their lifespan significantly [7, 8]. In the anhydrobiotic state, tardigrades are extraordinary tolerant to physical extremes including high and subzero temperatures [9-11], high pressure [9, 12], and extreme levels of ionizing radiation [13, 14]. Interestingly, tardigrades are even able to survive space vacuum (imposing extreme desiccation) and some specimens have even recovered after combined exposure to space vacuum and solar radiation [15]. Anhydrobiosis seems to be the result of dynamic processes and appears to be mediated by protective systems that prevent lethal damage. However, the molecular mechanisms of these processes are still poorly understood.



**Figure 1: SEM images of *M. tardigradum* in active state (I), tun state (III) and transition states (II, IV) between active and tun state.** Tardigrades are in the active (I) form when they are surrounded by at least a film of water. By losing most of their free and bound water (>95%) anhydrobiosis occurs. Tardigrades begin to contract their bodies (state II) and change their body structure into a so-called tun. Tardigrades in tun state (III) can be brought back to active state by rehydrating. SEM images have been provided by the cooperation partner: Dr. Ralph Schill, Steffen Hengherr, Zoological Department, University of Stuttgart.

## 1.2 FUNCRYPTA project and bioinspired products

Even though detailed aspects of the life cycle of tardigrades are described, there remains a notable absence of detailed analysis concerning the proteome and genome, which build the basics for further investigations including developmental analysis and also characterizing the molecular mechanisms of the protection processes in tardigrades. FUNCRYPTA (Functional Analysis of Dynamic Processes in Cryptobiotic Tardigrades) project consisting of four research

groups specialized in Zoology (Schill et al., Stuttgart), Genomics (Frohme et al., Wildau), Proteomics (Schnölzer et al., Heidelberg), Bioinformatics (Dandekar et al., Würzburg), and an industry cooperation partner (Oncoscience AG, Wedel) was established to fill this gap by performing a broad range of investigations and analyses.

Understanding the survival mechanisms in anhydrobiotic organisms will probably enable us to develop new strategies for preservation of biological macromolecules in the future, which is immensely important in medical field as well as in pharmaceutical industry. The need for stabilization of biological material in dried or in frozen state is particularly essential in organ and tissue preservation for transportation. Generally, expensive freezers are needed to transport e. g. vaccines and human blood platelets. Keeping vaccines or blood cold is challenging in particular in some parts of the world. Experts estimate that approximately half of all vaccinations are wasted annually due to contamination or exposure to extreme temperatures [16]. Eliminating the need for freezers by producing dry vaccines based on anhydrobiosis makes vaccines more available throughout the developing world. In October 2004 Cambridge Biostability came up with dry vaccines that reactivates once it is injected into the body [17]. The UK Department for International Development has awarded the company a £950,000 grant to bring the new vaccine to production. This technology involves embedding vaccine particles in sugar beads - known as sugar glasses - that can be stored without the need for refrigeration. These beads dissolve in the body to release the vaccine when injected. The accumulation of non-reducing disaccharide trehalose is reported to be associated with anhydrobiosis (see chapter 1.3). However the absence of trehalose in some organisms with excellent desiccation tolerance indicates the presence and involvement of other important biomolecules in protection mechanisms during anhydrobiosis. Their investigation and characterization could lead to revolutionary results in the field of preservation of biological material. Although achieving this goal requires years of research, we started the first steps of analyzing tardigrades that helped us to establish comprehensive resources for future investigations.

### **1.3 Involvement of disaccharides and proteins in anhydrobiosis**

It is very well known that desiccation and freezing are the extremes which damage cellular infrastructure leading to cell death. One of the most common osmoprotectants found in nature is the non-reducing disaccharide trehalose. By interacting with cell membranes trehalose prevents their disruption and furthermore proteins can also be protected from desiccation-induced denaturation by replacing water molecules [18]. It has been shown that trehalose in animals and sucrose in plants accumulate to high concentrations prior to dehydration in many anhydrobiotic organisms [19-21]. However, trehalose alone is not sufficient to confer a state of anhydrobiosis.

Particularly the absence of trehalose in organisms with excellent desiccation tolerance like *Bdelloid rotifers* and *Milnesium tardigradum* supports this view [22-24].

Furthermore a group of proteins, which are highly hydrophilic and thermostable have been reckoned to play an important role in anhydrobiotic animals. LEA (Late Embryogenesis Abundant) proteins have been described first in plant seeds [25, 26] and later in numerous organisms, including the nematodes *Caenorhabditis elegans* and *Aphelenchus avenae* [27], the bdelloid rotifers and the crustacean *Artemia franciscana* [28]. LEAs have been classified in at least six groups on the basis of their expression pattern and sequence [29]. Group 1, 2 and 3 are the most common LEA proteins and have been found unfolded in their native states. The first two groups could be detected only in plants, whereas group 3 is present in a variety of organisms. Even the exact function of LEA proteins is still unknown; experiments have shown that LEA proteins are involved in desiccation resistance in seeds, pollen, and anhydrobiotic plants [30].

Another protein group family that have been in focus in relation to anhydrobiosis is the heat shock protein (Hsp) family. Hsps are molecular chaperones that protect intracellular macromolecules against unfolding and aggregation. The extraordinary stress resistance of encysted *Artemia* embryos is described to depend on accumulation of small heat shock protein (sHsp) p26 [31, 32] and the ferritin homologue artemin [33, 34]. Furthermore Hsp70 is suggested to be involved in repair processes after desiccation in tardigrades [16].

Whereas LEAs and Hsps are associated with anhydrobiosis in particular because of their chaperone activity, there are reports of regulation of proteins involved in “desiccation-avoidance strategy” [4]. Aquaporins (AQPs) are passive transport channels for water and permit water to move in the direction of an osmotic gradient. Kikawada et al. analyzed two AQPs of *Polypedilum vanderplanki*, one of the largest anhydrobiotic animals [4]. The result shows that one AQP controls the water homeostasis of fat bodies during normal conditions whereas the other one is involved in the removal of water in the desiccation process en route to anhydrobiosis.

The absence of trehalose in *M. tardigradum* and the association of proteins such as LEAs with anhydrobiosis lead to the urgent need for investigating the proteome of tardigrades in regard to identifying and characterizing other proteins that probably have important roles in protection mechanisms during anhydrobiosis.



## 1.4 Analyzing the proteome of tardigrades using high throughput proteomics technologies

### 1.4.1 Proteome profiling

Proteomics is a rapidly expanding field that is applied for protein profiling, analyzing the protein functions, interactions and dynamics. The improvement of proteomics technologies for answering complex biological questions led to the development of increasingly sensitive qualitative and quantitative methods. Mass spectrometry is a versatile and indispensable tool in proteomics. The mass-to-charge ratio ( $m/z$ ) of peptides, which are generated by electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) is determined by mass spectrometry and tandem MS (MS/MS) enables the sequencing of peptides. The sensitivity and accuracy of modern mass spectrometers allow the analysis of nanograms of individual proteins and the proteolytically generated peptides thereof. The mass error is instrument dependent and is often less than 10 ppm.

Proteomic approaches are used to systematically analyze the proteins expressed by cells or tissues to generate comprehensive proteome reference maps as has been shown for different organisms. First-generation large-scale proteome maps of microorganisms such as yeast or the bacterium *Deinococcus radiodurans* are examples of such projects [35].

As proteomics partner of the FUNCRIPTA project we aimed to establish a comprehensive proteome resource for *M. tardigradum* employing optimized protocols for protein extraction, generation of high-resolution gels and high-throughput protein identification by electrospray ionization tandem mass spectrometry (ESI-MS/MS). The classical method of protein identification is based on homology search using a protein database. The major limitation of this method is dependency to a protein database which should be ideally complete. Since at the beginning of the project no specific protein database for tardigrades was available and only 12 proteins originating from *M. tardigradum* were recorded in the publicly available NCBI nr database our genomic cooperation partner initiated a sequencing project to develop a tardigrade specific EST database, which was translated to a protein database. Although database search using NCBI nr delivered identification of highly conserved proteins like actin or some heat shock proteins, the majority of spectral analyses yielded no positive characterization. By using the tardigrade specific database in combination with high sensitive proteomics approaches it was possible to analyze more than 3000 unique proteins and to develop the first proteome resource of tardigrades that provides the basis for further studies utilizing sensitive quantification techniques in order to understand important physiological processes such as anhydrobiosis and stress resistance.

### 1.4.2 Quantitative analysis of tardigrades in three different states

In addition to general profiling of the proteins present in a sample (in our case whole lysate of an organism), information on the level of protein expression in different states is required to understand specific molecular mechanisms. The development of accurate quantification methods is currently one of the most challenging area of proteomics. The selection and design of quantification experiments is dependent on multiple factors including the source and the number of the samples, the type of available instrument and furthermore expense and time [36]. Two types of quantitative approaches are available: absolute and relative quantification. Whereas absolute quantification determines the exact concentration of each protein present in the sample, relative quantification determines the up- or down-regulation of a protein relative to the control sample. The quantification methods can be a) labeling-based (isotope labeling and fluorescent labeling) or b) label-free. Fluorescent labeling is mostly used for 2D gels (two-dimensional fluorescence difference gel electrophoresis, 2D DIGE). DIGE technology is also performed successfully in combination with blue-native (BN) gel system for analyzing membrane proteins [37]. Isotope labeling methods are generally combined with 1D gels as a fractionation alternative of complex samples. Isotope-Coded Affinity Tag (ICAT) [38], Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) [39],  $^{15}\text{N}/^{14}\text{N}$  metabolic labeling [40],  $^{18}\text{O}/^{16}\text{O}$  enzymatic labeling [41], Isotope Coded Protein Labeling (ICPL) [42], Isobaric Tags for Relative and Absolute Quantification (iTRAQ) [43] belong to isotope labeling methods. Most labeling-based quantification approaches have potential limitations [44]. Increased complexity of sample preparation, incomplete labeling, high sample concentration and high cost of the reagents are the most important aspects. Furthermore some technologies such as SILAC and  $^{15}\text{N}/^{14}\text{N}$  metabolic labeling rely on metabolic incorporation of the isotopes and are suitable for cell culture and only in rare cases for whole organisms especially because the whole food chain of the organism has to be considered for labeling. Therefore the interest for label-free techniques, which allow faster and simpler quantification, has been increased.

Label-free approaches are generally based on measurements of ion intensity changes like peptide peak areas and peak heights in chromatography, or counting the spectra of identified proteins after MS/MS analysis [44]. Both methods are relative quantification approaches. In the spectral counting method the number of identified MS/MS spectra from the same protein in each individual LC-MS/MS dataset is calculated and used to show the changes in protein expression by direct comparison. This can be explained by the fact that increase in protein abundance results in an increase in the number of detected proteolytic peptides, which induces the increase of number of identified unique peptides and protein sequence coverage [44, 45]. It has been shown that only spectral count is in linear correlation with relative protein abundance [46], and therefore is a simple and reliable index for relative protein quantification [47, 48]. However,

normalization and statistical analysis is needed for accurate and reliable detection of changes in protein expression for these two approaches [44, 49]. Another label-free approach is based on Protein Abundance Index (PAI), defined as the number of identified peptides divided by the number of theoretically observable peptides for each protein. This index was later converted to exponentially modified PAI (emPAI, the exponential form of PAI minus one) [50]. The success of using emPAI was demonstrated by determining absolute abundance of 46 proteins in a mouse whole-cell lysate, which had been measured using synthetic peptides. The emPAI can be directly used for reporting approximate protein abundance in a large-scale analysis as shown in different studies [49, 51-54].

In the present study a semi-quantitative analysis of proteins expressed in different states was performed by calculating emPAI. Using this method, we compared firstly the proteome of tardigrades in early embryonic state *versus* adult tardigrades (in both active and tun state). This analysis delivered knowledge about proteins associated with embryonic development, which is of major importance in the field of developmental biology. Secondly, we performed semi-quantitative analysis of differentially expressed proteins in active *versus* tun state, which resulted in new aspects in investigating anhydrobiosis and protection mechanisms. For quantitative analysis of proteins in active *versus* tun state we alternatively employed 2D DIGE technology that allows reducing the complexity of analysis, since individual protein spots showing up- or down-regulation on 2D gels can be selected and analyzed.

## 2 Materials and methods

### 2.1 Materials

The chemicals have been ordered in p. a. quality from following companies:

Fluka (Steinheim), Carl Roth GmbH (Karlsruhe), GE Healthcare GmbH (Freiburg), Promochem GmbH (Wesel), Sigma-Aldrich Chemie (Steinheim), Novagen (Gibbstown, USA), Mallinckrodt-Baker (Deventer, Niederlande), Merck Biosciences (Darmstadt), Gerbu Biochemica GmbH (Gaiberg), VWR (Darmstadt), Invitrogen GmbH (Karlsruhe), Promega GmbH (Mannheim), and Thermo Fisher Scientific (Bonn).

Buffers have been prepared using ultrapure water (ddH<sub>2</sub>O) filtered by a Millipore Instrument (Millipore GmbH, Schwalbach/Ts.) as described before [55, 56]. Buffers and solutions for performing 2D DIGE electrophoresis were prepared as described in Ettan DIGE User Manual (18-1164-40 Edition AA, GE Healthcare).

#### Instruments

Biofuge fresco	Thermo Fisher Scientific, Bonn
CapLC nanoHPLC System	Waters GmbH, Eschborn
Ettan DALTwelve	GE Healthcare GmbH, Freiburg
Ettan IPGphor	GE Healthcare GmbH, Freiburg
Labofuge 400 "Function Line"	Thermo Fisher Scientific, Bonn
LQT-Orbitrap XL	Thermo Fisher Scientific, Dreieich
Microplate Reader EL 800	BioTek Instruments Inc., Winooski, USA
QTOF Ultima	Waters GmbH, Eschborn
Speed Vac	Thermo Fisher Scientific, Bonn
Thermomixer comfort	Eppendorf AG, Hamburg
Typhoon 9410	GE Healthcare GmbH, Freiburg
Ultraflex I	Bruker Daltonik GmbH, Bremen

#### Softwares

BioTools 3.0	Bruker Daltonik GmbH, Bremen
Blast2GO	<a href="http://www.blast2go.org/">http://www.blast2go.org/</a>
DeCyder 2D 6.5	GE Healthcare GmbH, Freiburg
EndNote 11 und 12	Thomson Reuters, Carlsbad, USA
FlexAnalysis 2.4	Bruker Daltonik GmbH, Bremen
FlexControl 2.4	Bruker Daltonik GmbH, Bremen
ImageQuant Tool 5.2	GE Healthcare GmbH, Freiburg
KCjunior 1.1	BioTek Instruments Inc., Winooski, USA
Mascot 2.1 und 2.2	Matrix Science, London, UK
MassLynx 4.2	Waters GmbH, Eschborn
Office 2003 und 2007	Microsoft, Redmond, USA
Protein Lynx Global Server 2.2.5	Waters GmbH, Eschborn
Typhoon Scanner Control 5.0	GE Healthcare GmbH, Freiburg

## 2.2 Methods

### 2.2.1 From tardigrade sampling to proteome analysis

The detailed information about following experimental methods used in the present study can be found in both accepted publications [55, 56].

- Tardigrade culture and sampling
- Sample preparation
- 1D gel electrophoresis
- 2D gel electrophoresis
  - Strip loading
  - Isoelectric focusing
  - Second dimension (SDS PAGE)
  - Gel staining
- Western blotting
- Protein identification
  - In-gel digestion
  - Peptide extraction
  - ESI-MS/MS analysis and database search
  - Generation of databases
- Classification of proteins
- Protein domain analysis
- Multiple alignments and secondary structure prediction

Other methods that are not included in the publications are presented in the following chapters.

### 2.2.2 Sampling of tardigrades in early embryonic state

Eggs in the early embryonic state (blastula state), according to Suzuki [3] were selected for the analysis. During the egg deposition that is always accompanied by a moult process eggs are laid inside the old cuticle. The average clutch contains about 7 eggs with a minimum of 3 and a maximum of 12. The egg laying process usually takes less than two minutes from the first to the last egg. Egg containing cuticles (780 eggs in total) were collected 24 h after egg deposition, washed several times with Volvic™ water. All samples were frozen in liquid nitrogen and stored at -80°C. Eggs (blastula, 780 eggs) were homogenized in 60 µl lysis buffer as described before [56].

### 2.2.3 Sample preparation for phosphoprotein analysis

Animals (200 individuals each for active and tun state) and eggs (blastula, 780 eggs) were homogenized as described before [56], with the slight modification of adding phosphatase inhibitors to the lysis buffer. Briefly, collected animals/eggs were homogenized in 60  $\mu$ l lysis buffer containing 8M urea, 4% CHAPS, 30 mM Tris, Protease Inhibitor Mix (GE Healthcare), Phosphatase inhibitor cocktail 1+2 (Sigma) and Orthovanadate (50mM), pH 8,5 by ultrasonication (SONOPULS, HD3100, Bandelin Electronic) with 45% amplitude intensity and 1-0.5 sec intervals at 4°C. Orthovanadate (50 mM) has been prepared as described by Thingholm et al. [57]. 20  $\mu$ l of each phosphatase inhibitor cocktail 1+2 and orthovanadate (50 mM) have been added to 1 ml lysis buffer to inhibit phosphatase activity. After homogenization samples were shock frozen and stored at -80°C.

### 2.2.4 Two-dimensional fluorescence difference gel electrophoresis (2D DIGE)

#### Sample preparation and protein labeling with CyDye DIGE fluor

Whole protein lysate of tardigrades in active and tun state have been prepared as described before [56]. To perform 2D DIGE we pooled the protein lysate of 1600 tardigrades in each active and tun state. Protein concentration was determined using the BCA assay kit. The protein lysates were labeled with Cy2, Cy3, and Cy5 following the protocols described in the Ettan DIGE User Manual (18-1164-40 Edition AA, GE Healthcare). The DIGE experimental design is shown in Table 1. Typically, 50  $\mu$ g of lysates were labeled with 400 pmol of Cy3 or Cy5, while the same amount of the pooled standard that contained equal quantities of all the samples was labeled with Cy2. Labeling reactions were carried out in the dark for 30 min on ice before quenching with 1 mL of 10 mM lysine for 10 min on ice. These labeled samples were then combined for 2D DIGE analysis. Samples for 6 technical replicates and one preparative gel have been prepared.

**Table 1: Setup of DIGE experiment for relative quantification of proteins in active and tun state.**

Number of gels	Labeling with		
	Cy2	Cy3	Cy5
1. Gel	Internal Standard	AS Replicate 1	TS Replicate 1
2. Gel	Internal Standard	TS Replicate 2	AS Replicate 2
3. Gel	Internal Standard	AS Replicate 3	TS Replicate 3
4. Gel	Internal Standard	TS Replicate 4	AS Replicate 4
5. Gel	Internal Standard	AS Replicate 5	TS Replicate 5
6. Gel	Internal Standard	TS Replicate 6	AS Replicate 6

## 2D gel electrophoresis and image acquisition

2D gel electrophoresis was performed as described earlier with the modification of performing all steps in the dark and using low-fluorescent glass plates for the second dimension [56].

The Cy2, Cy3, and Cy5-labeled images were acquired on a Typhoon 9410 scanner at the excitation/emission wavelengths of 488/520, 532/580, 633/670 nm, respectively.

## Image analysis

DeCyder v.6.5 was used to analyze the DIGE images as described in the Ettan DIGE User Manual (GE Healthcare).

Intragel spot detection and quantification and intergel matching and quantification were performed using differential in-gel analysis (DIA) and biological variation analysis (BVA) modules, respectively. Briefly, in DIA, the Cy2, Cy3, and Cy5 images for each gel were merged, spot boundaries were automatically detected, and normalized spot volumes (protein abundance) were calculated. The resulting spot maps were exported to BVA. The best internal standard image was assigned as the “Master”, which was used as a template. The protein spots on the remaining internal standard images were all matched to the master gel to ensure that the same protein spots were compared between gels. Matching of the protein spots across all gels was performed after several rounds of extensive land marking and automatic matching. Finally the match was checked manually to ascertain the accuracy of the match process. Dividing each Cy3 or Cy5 spot volume with the corresponding Cy2 (internal standard) spot volume within each gel gave a standard abundance, thereby correcting intergel variations. Statistical analysis was performed using Student’s T-test. We have selected a protein filter with the following parameter to determine the protein spots of interest: Student’s T-test  $<0.05$  and average ratio  $>1.25$  or  $<-1.25$ , appearance in more than 75% of gel images.

### 2.2.5 Phosphoprotein detection

2D gel electrophoresis was performed as described before with slight differences. We loaded 50  $\mu\text{g}$  protein and used 13 cm long IPG strips with non-linear gradients from pH 3–11. For the isoelectric focusing (IEF) in the first dimension the following running protocol was used: 9 h at 500 V, 1-h gradient up to 1000 V, 2.5-h gradient up to 8000 V and 10 min at 8000 V.

Gels were immersed in 250 ml ultrapure water for 10 min immediately after the electrophoresis. All steps have been performed by gentle agitation using an orbital shaker (35 rpm) in the dark. After a second wash step with ultrapure water, gels were incubated in fixation solution (50% methanol, 10% acetic acid) at least twice for 30 min and overnight. The gels were then washed

with 250 ml ultrapure water (three changes, 30 min *per* wash). The Pro-Q Diamond phosphoprotein staining solution was prepared by diluting the stock solution three fold with water. The gels were incubated with 150 ml staining solution for 2 h in the dark, and destained with 250 mL of destaining solution in the dark (four changes, 30 min *per* wash). The gels were washed twice with ultrapure water for 5 min each. The images were acquired on Typhoon 9410 with a 532 nm laser excitation and a 580 nm bandpass emission filter. Following the image acquisition, gels were post-stained with SYPRO Ruby overnight as described in the staining protocol (Molecular Probes).



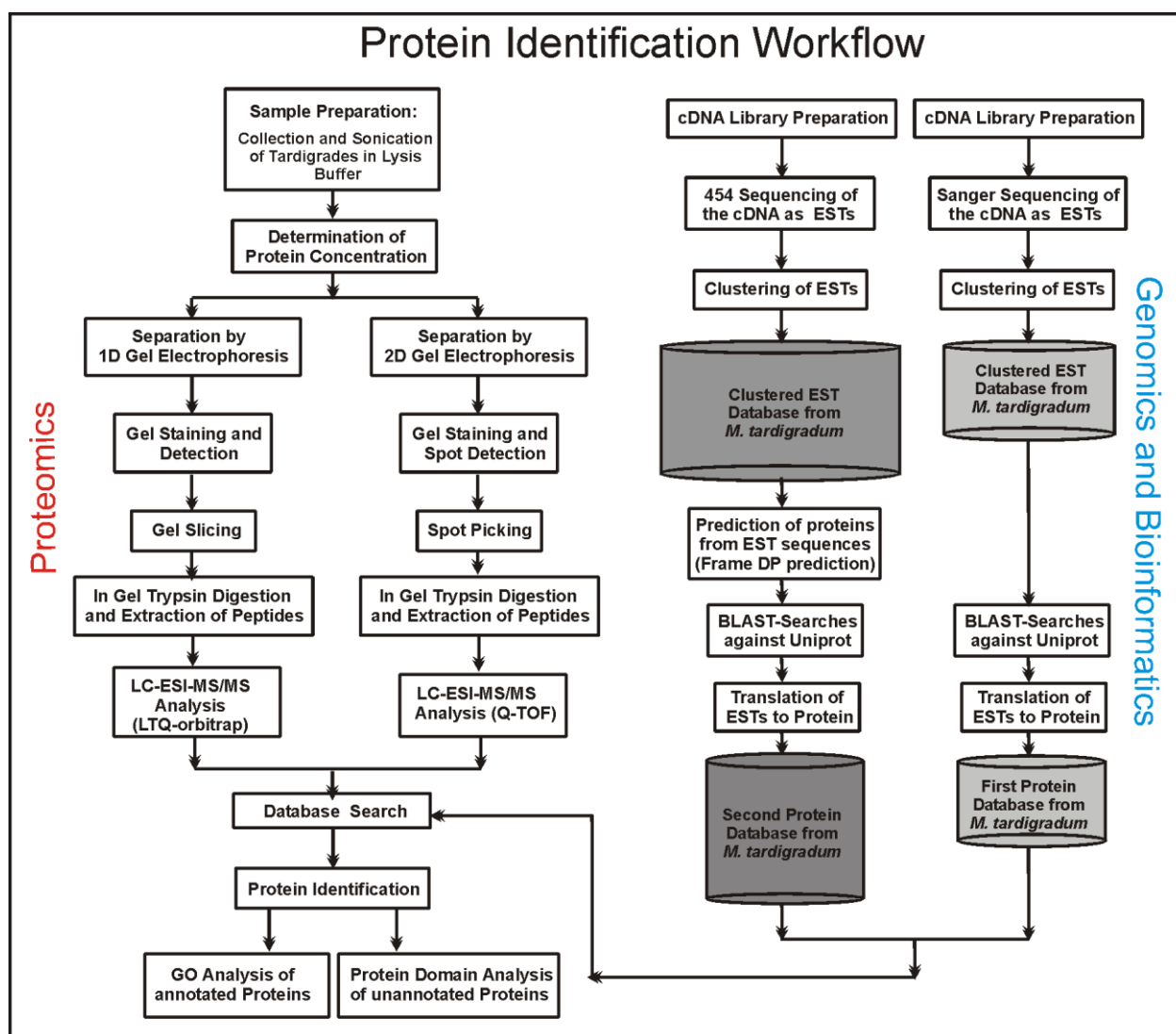
## 3 Results and discussion

### 3.1 Comprehensive proteome profiling of *M. tardigradum*

#### 3.1.1 Developing a 2D proteome map

To establish and optimize a reliable and robust protocol for the extraction of proteins from whole tardigrades we applied different workup protocols and evaluated them by one-dimensional (1D) gel electrophoresis [56]. We could show that direct homogenization of tardigrades in lysis buffer without any previous precipitation steps is most efficient and enables the generation of high quality 2D gels. In particular the selected extraction protocol is suitable for 2D DIGE experiments. Since nothing was known about the proteolytic activity in *M. tardigradum* special precautions were taken to avoid any protein degradation or proteolysis throughout the whole workup procedure. Integrity of proteins was analyzed by Western blot analysis of two housekeeping proteins actin and tubulin [56]. Since these two proteins are highly conserved, the sequence homology was assumed to be high enough to detect the proteins with commercially available antibodies. The development of a robust workup protocol laid the basis for the generation of a protein map from whole tardigrades in the active state.

Identification of proteins depends on the representation of the sequence or a close homologue in the database. When we started our study of the tardigrade proteome very little was known about tardigrades at the genome and gene expression level. Only 12 proteins were recorded in the NCBI nr database, which originate from *M. tardigradum*. For all of them only partial sequences ranging from as few as 43 amino acids for beta actin up to 703 amino acids for elongation factor-2 were available. Since the presence of a comprehensive protein database is a prerequisite for protein identification, a *M. tardigradum* EST sequencing project has been initiated in parallel to our proteomic study by our genomic cooperation partner (Frohme et al.). Subsequently, two tardigrade specific protein databases have been established as shown in Figure 2. The first protein database is based on Sanger sequences and contains 3318 protein sequences, the second protein database is based on 454 sequences and contains 24679 protein sequences.



**Figure 2: The experimental workflow to analyze the proteome of *M. tardigradum*.** The proteome analysis of tardigrades was performed using two complementary proteomics platforms; one-dimensional (1D) and two-dimensional (2D) gel electrophoresis. The results of 2D gel electrophoresis en route to developing a proteome map are already published [56]. The current study (submitted to Proteomics) is based on 1D gel analysis in combination with high sensitive nanoLC ESI-MS/MS on a LTQ-Orbitrap mass spectrometer. Tardigrades were homogenized directly in lysis buffer. Total protein extracts of tardigrades in early embryonic state and adult tardigrades in active and tun state were separated by 1D gel electrophoresis. After Coomassie staining protein bands were sliced and in-gel digested with trypsin. MS/MS data obtained by LC-ESI-MS/MS analysis were searched against the tardigrade specific database. The database has been developed by translating EST sequences of *M. tardigradum*, which were obtained by 454 sequencing. Identified proteins with annotation were classified in different functional groups using the Blast2GO program. Identified proteins without annotation were analyzed with the DomainSweep program to search for specific protein domains. The current study serves as a comprehensive protein resource of tardigrades and in addition demonstrates a comparative analysis of proteins expressed in different states. The genomics and bioinformatics steps have been performed by our cooperation partners; Prof. Frohme, Wildau and Prof. Dandekar, Würzburg.

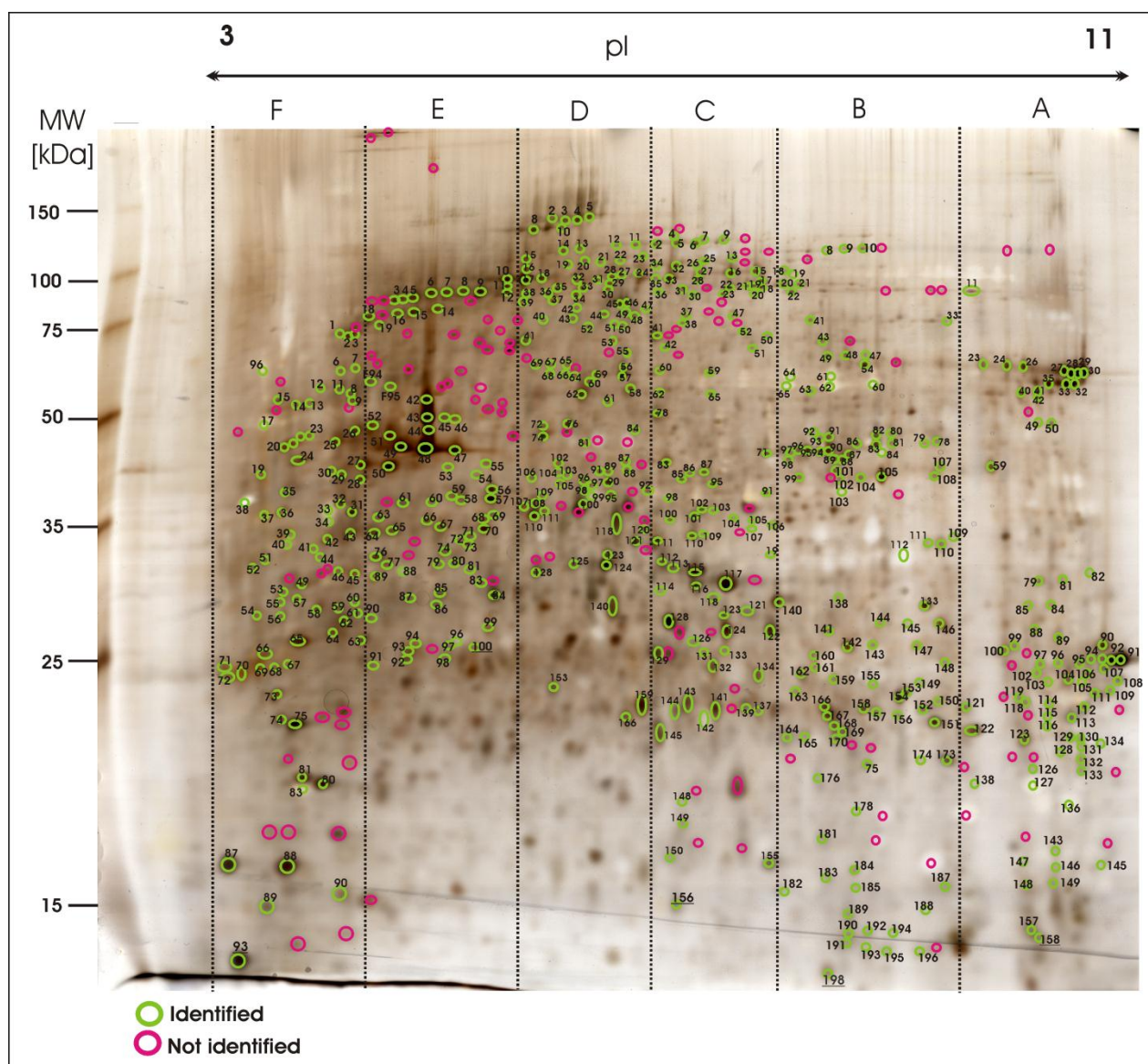
As the first step in the tardigrade's project we began to develop a proteome map of tardigrades in active state utilizing 2D gel electrophoresis and LC-MS/MS analysis [56], as shown in experimental workflow in Figure 2. 2D gel electrophoresis allows the separation of complex protein mixtures according to two completely independent physico-chemical parameters of

proteins (pI in the first dimension and MW in the second dimension) and therefore offers high resolution. Furthermore single spots, which contain only up to a few proteins can be analyzed by mass spectrometry separately and this reduces the complexity of the protein analysis. In particular the absence of a comprehensive database makes the reduction of complexity necessary. The first protein database was prepared using Sanger sequences, which were originated from a cDNA library of tardigrades in different states (active, inactive, transition states). The cDNAs were sequenced as ESTs and clustered. Thereby, we obtained a nucleotide database containing 818 contigs and 2500 singlets. The clustered EST database was translated in a protein database by homology search against Uniprot protein database. Using this database we analyzed 606 protein spots of a preparative 2D gel of whole protein lysate of adult tardigrades in active state en route to developing the first proteome map of tardigrades. The selected 606 spots from the 2D gel correspond to some highly expressed proteins, but mostly to spots in the medium and low expression range. The MS/MS data generated by LC-MS/MS on a QTOF instrument were searched against the tardigrade protein database as well as against publicly available NCBI nr database to show the advantage of the tardigrade protein database. The comprehensive analysis of database searches is available in the publication Schokraie et al. [56]. Briefly, 56 unique proteins were successfully identified by searching the NCBI nr database. It concerns proteins which are either highly conserved among different species e. g. actin or protein entries from *M. tardigradum* which are already available in the NCBI nr database e.g. elongation factor 1-alpha. Further 73 unique proteins could be identified by searching the tardigrade protein database and another 15 unique proteins were present in both databases. Furthermore 150 spots were identified as proteins with yet unknown functions. In summary, 421 (69.5 %) out of 606 protein spots which were picked from the preparative 2D gel were identified. 271 spots yielded 144 unique proteins with distinct functions whereas 150 identified spots correspond to 36 unique proteins without annotation. For these proteins of unknown function more information could be obtained by applying protein domain annotation methods. We ran all proteins through the DomainSweep pipeline which identifies the domain architecture within a protein sequence and therefore aids in finding correct functional assignments for uncharacterized protein sequences. It employs different database search methods to scan a number of protein/domain family databases. 2 out of the 36 unique proteins gave a significant hit, whereas 28 proteins were listed as putative and 6 proteins gave no hit at all. The result of this analysis is included in Supplementary Table 3 of publication [56].

Since our tardigrade EST project provided us recently with a new protein database containing a high number of 24679 protein sequences generated by 454 sequencing, we updated the result of 2D gel analysis by performing the database search against this new database. A total number of 502 (82.8%) protein spots had significant protein hits. The corresponding protein spots are indicated by green circles in the 2D reference map shown in Figure 3. 332 unique proteins have

been identified, from which 133 were identified by more than one significant peptide. The remaining 199 proteins could be identified by one significant peptide. A total number of 81 unique proteins are without annotation and were analyzed by searching specific protein domains. Detailed information about each of the identified proteins including spot number, protein annotation, accession number (tardigrade specific accession number), total protein score, number of matched peptides, and sequence coverage is listed in Supplementary Table 1. Since the new database contains a high number (24679) of protein sequences we could identify most of selected protein spots (82.8%). Proteins that could not be identified are mostly of low expression range as it is shown in Figure 3. In particular tardigrade specific proteins which could not be identified before are present now in the result; e. g. major egg antigen and protein members of large lipid transfer protein superfamily like vitellogenins have been identified for the first time in tardigrades.

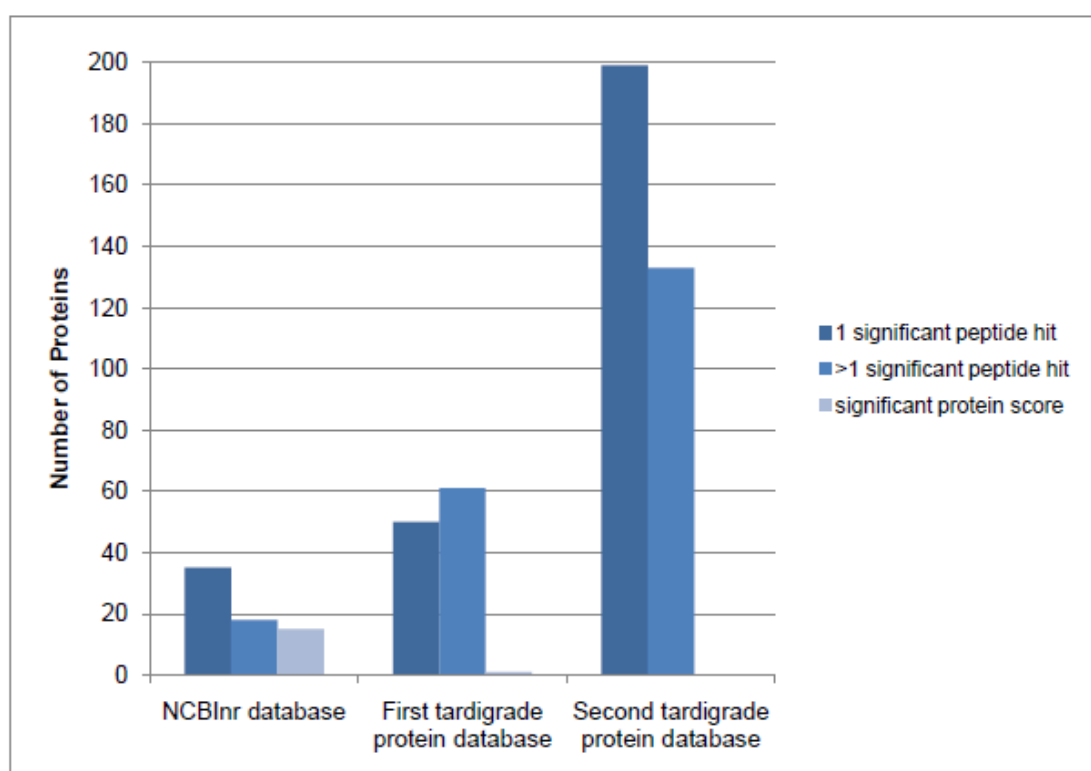
The localization of spots on the 2D map delivers information about the MW, pI and posttranslational modifications (PTM) for instance phosphorylation. Vitellogenin is a phospholipo-glycoprotein which functions as a nutritional source for the development of embryos [58]. During developing oocytes vitellogenin and vitellin are modified through cleavage and by different PTMs like glycosylation and phosphorylation. Interestingly we could identify vitellogenin in several spots on the 2D gel showing vertical (pI) shifts most probably caused by PTMs.



**Figure 3: Image of a preparative 2D gel with selected analyzed protein spots.** Total protein extract of 400 tardigrades in the active state corresponding to 330  $\mu$ g was separated by high resolution two-dimensional gel electrophoresis. Proteins were visualised by silver staining. Two different categories are shown: Identified proteins with functional annotation are indicated in green and not yet identified proteins are indicated in red.

When we examined the protein hits obtained by the three databases in more detail we found that in the NCBI database approximately one half of the proteins were identified by only one significant peptide hit. For about 25% of the proteins more than one significant peptide hit was obtained (Figure 4). For the remaining 25% only the protein score which is the sum of two or more individual peptide scores was above the significance threshold while none of the peptide scores alone reached this value [56]. In contrast, proteins found in the Sanger tardigrade protein database were predominantly identified by more than one significant peptide hit whereas a smaller number was represented by only one peptide. These results are not surprising. Since the NCBI database contains very few sequences originating from *M. tardigradum* e.g.

elongation factor 1-alpha the identification relies predominantly on high homologies between tardigrade sequences and sequences from other more or less related species of other taxa. The chances for detecting more than one identical peptide is significantly higher when searching MS/MS data against the tardigrade protein databases since these databases contain only tardigrade specific sequences. Database search against 454 database delivered a high number of proteins identified with more than one peptide, which is approximately 2fold higher comparing to database search against Sanger database. However, the number of one-peptide identifications is also high, which is due to the short protein sequences present in the 454 database.

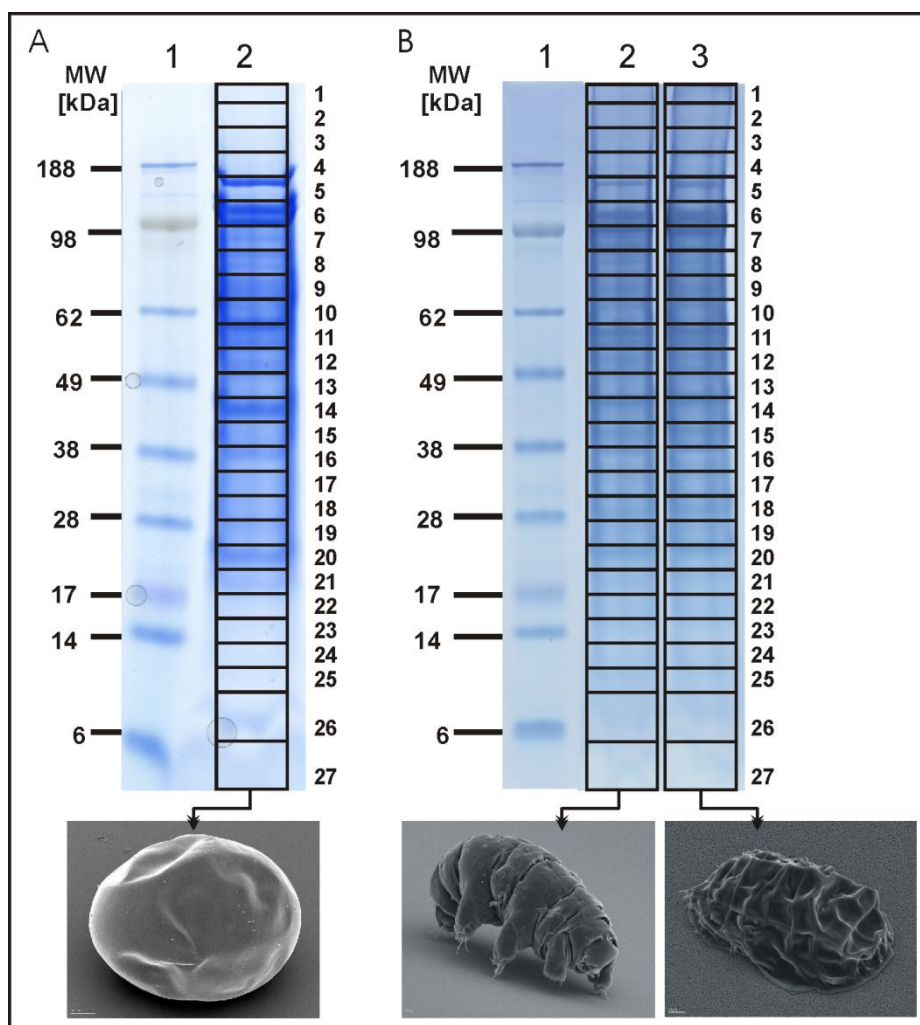


**Figure 4: Statistical analysis of significant peptides found in the three different databases which were used to search the MS/MS data.** The number of significant peptide hits is compared between the different databases. When searching against the NCBI database only a few proteins mostly with only one significant peptide hit could be identified. The first tardigrade protein database contains 3318 proteins translated from ESTs generated by Sanger sequencing. Using this database most proteins were represented by two or more significant peptides. The second tardigrade database is based on EST sequences generated by 454 sequencing and contains a high number of protein sequences (24679). Accordingly the identification rate was extremely increased.

### 3.1.2 Proteome analysis using 1D gel electrophoresis

The high number of EST sequences generated by 454 sequencing made it possible to consider the 1D gel electrophoresis as a complementary platform to 2D gel electrophoresis to analyze the proteome of tardigrades (Figure 5). 1D gel electrophoresis in combination with high sensitive nanoLC ESI-MS/MS on a LTQ-Orbitrap mass spectrometer allows protein identification in a

large scale. Using this approach we analyzed the proteome of *M. tardigradum* in three different states: early embryonic state (EES), adults in active state (AS) and tun state (TS). The whole protein lysate of early embryonic state and adult tardigrades in active and tun state were separated on a 1D gel and were cut into 27 slices as shown in Figure 5. Peptides were obtained by in-gel tryptic digestion and extraction of single slices separately.



**Figure 5: Separation of whole protein lysate of tardigrades in early embryonic state and adult tardigrades in active and tun state on one-dimensional gel.** Total protein extract of tardigrades in early embryonic state and adult tardigrades in active and tun state have been separated on one-dimensional polyacrylamide gel. A) Lane 1: Rainbow molecular weight marker. Lane 2: Protein extract of whole tardigrades in early embryonic state. B) Lane 1: Rainbow molecular weight marker. Lane 2: Protein extract of whole adult tardigrades in active state. Lane 3: Protein extract of whole adult tardigrades in tun state. Corresponding SEM-images for tardigrades in each state is included (provided by the cooperation partner: Dr. Ralph Schill, Steffen Hengherr, Zoological Department, University of Stuttgart).

The analysis yielded 1982 unique proteins in EES, 2345 unique proteins in AS and 2281 unique proteins in TS. Whereas in our first publication (using Sanger sequences) many proteins have been identified with only one significant peptide, in this study all proteins are characterized by at least two significant peptides and one peptide identifications are generally not considered for further analysis. The result of database search and protein identification for each state is

included in the manuscript submitted to Proteomics. In the Supplementary Table 2 identified proteins with annotation are listed and their expression level in three different states is compared using emPAI. By setting the search parameters as such that they refer to a match probability of  $p < 0.01$ , we minimized the false discovery rate (FDR) dramatically (below 5%).

This current study presents a comprehensive proteomic resource of *M. tardigradum* considering different life states. We identified more than 3000 unique proteins of *M. tardigradum* with high sequence coverage. Database search of MS/MS spectra resulted in proteins, which fall into two groups: identified proteins with annotation and identified proteins without annotation. Identified proteins with annotation (2460 unique proteins) were classified into different functional groups defined by gene ontology using Blast2GO program. In addition, 658 tardigrade specific proteins have been identified, which could not be annotated by homology search and therefore have been analyzed by searching for specific protein domains. In summary, the proteome analysis resulted in identification of proteins involved in a broad range of biological processes. In particular chaperones (e.g. hsp), antioxidant proteins (e.g. GSTs and SODs), aquaporins, and LEA proteins are of major interest since they have been reported to be regulated in other anhydrobiotic organisms.

## **Key proteins related in anhydrobiosis and survival**

### **Late embryogenesis abundant protein**

Among the known proteins that are associated with anhydrobiosis we identified protein members of LEA (Late Embryogenesis Abundant) family. Although the precise role of LEA proteins has not yet been fully elucidated, different groups have reported on their association with tolerance to water stress by desiccation [29], [59]. LEA protein of group 3 could already be identified in nematodes *C. elegans*, *Steinernema feltiae* and *Aphelenchus avenae*, and the prokaryotes *Deinococcus radiodurans*, *Bacillus subtilis* and *Haemophilus influenzae* [26], [27, 60]. The presence of LEA proteins in tardigrades has been shown by proteomic analyses of 2D gels prepared from *M. tardigradum* whole lysate [56]. The identification of LEA protein was previously performed by searching the MS/MS data against NCBI database. In the present study LEA is characterized for the first time by MS/MS sequencing from *M. tardigradum* database. Contig00574:89:529:2 shows high similarity to the LEA protein from *Alteromonas macleodii*. The predicted sequence from *M. tardigradum* was confirmed by peptides sequenced by MS/MS covering 61.9% of the entire sequence. We analyzed six other contigs (probably belonging to LEA protein family) by DomainSweep analysis to search for specific protein domains as shown in Table 2. For 5 contigs (02113:1:319:2, 19683:1:923:2, 01844:1:445:1, 22151:1:238:2, 25946:1:607:2) Blast search delivered hits for LEA proteins, but we could not consider the result as significant because of insufficient e-value. For one contig (23719:1:474:2) we did not receive any Blast result at all. DomainSweep analysis delivered two contigs as significant hits and 4 contigs as putative candidates for LEA proteins as shown in Table 2.



Comparison of the emPAI of significantly annotated contig (00574:89:529:2) as LEA protein, shows that LEA protein is up-regulated in tun state. However, this result has to be validated using orthogonal quantitative methods.

**Table 2: Identified proteins that are significant or putative candidates for LEA proteins.**

Protein annotation	emPAI			Domain Sweep analysis
	EES	AS	TS	
contig00574:89:529:2  LEA protein [Alteromonas macleodii ATCC 27126] (ZP_04714463.1) Evalue: 4e-05	10.19	15.19	16.8	
contig02113:1:319:2 No Annotation	1.5	5.47	2.4	significant
contig19683:1:923:2 No Annotation	/	/	0.1	significant
contig01844:1:445:1 No Annotation	4.15	4.36	3.84	putative
contig22151:1:238:2 No Annotation	9.13	11.15	7.37	putative
contig25946:1:607:2 No Annotation	0.32	1.02	1.43	putative
contig23719:1:474:2 No Annotation	0.63	1.72	1.05	putative

## Chaperones

The major protein family with high importance among the proteins with chaperone function is heat shock protein (Hsp) family. A comprehensive comparative proteomic study of Hsps in tardigrades in active *versus* tun state has been reported earlier [55]. Protein members of different heat shock protein families are present in our results: Hsp90, Hsp70, Hsp60, Hsp40 and Hsp20 families and furthermore GroES and GrpE families. We have found two protein sequences, which have been annotated as major egg antigen that belongs to the small heat shock protein (Hsp20) family. Major egg antigen (p40) is found in *Schistosoma mansoni* and is described to be involved in response to heat.

In addition to the Hsps described earlier [55] we found three sHsps that are described for the first time in *M. tardigradum*: the small heat shock protein C4 and 10kDa heat shock protein (GroES chaperonin family) present only in EES and a sHsp (AGAP000941-PA, sHsp 20.6 isoform 3 (contig01971:138:399:3)) only in adults. Hsps are involved in catalyzing the refolding of denatured proteins or folding of newly synthesized proteins and preventing protein aggregation [61]. The relation of Hsps in particular low molecular weight Hsps in desiccation tolerance and dormancy is reported in different studies [31, 62]. Using the label-free quantification based on emPAI we have performed a semi-quantitative analysis of Hsps expressed in tardigrades [55]. The comprehensive analysis and discussion included in publication [55] can be found in the chapter 8.2. Briefly, in parallel to the gene expression analysis of heat shock proteins in *M. tardigradum* [63] we found a member of Hsp90 family up-regulated in the anhydrobiotic state. Furthermore the most abundant protein members of Hsp70 family are down-regulated in the anhydrobiotic state, which is in agreement with the observations in *R. coronifer*. Since up-regulated Hsp70 proteins in the anhydrobiotic state are generally low abundant, we conclude that they contribute only a small part of the Hsp70 contingent in the cell. The investigation of sHsp regulation in diapausing organisms indicates a

broad range of functions. Gene expression studies of some selected heat shock proteins in *M. tardigradum* showed the up-regulation of one sHsp by heat-shock treatment and the down-regulation of another sHsp in the transition state from the anhydrobiotic to the active state [63]. However, the role of these two sHsps in anhydrobiosis is not yet clear. In our investigation the most abundant heat shock protein in tardigrades in all three states and in particular in EES is the small heat shock protein major egg antigen (p40).

### **Antioxidant proteins**

Living organisms are permanently exposed to non-nutritional foreign chemical species (xenobiotics). In addition, the presence of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical, which are produced as toxic byproducts of normal cellular metabolism (endobiotics), makes the existence of detoxification systems essential for surviving. Superoxide dismutases (SODs) are one of the most important antioxidant enzymes in defense against ROS and particularly superoxide anion radicals [64, 65]. Generally SOD is present in two forms inside the eukaryotic cell, SOD (Cu-Zn) in the cytoplasm and outer mitochondrial space, and SOD (Mn) in the inner mitochondrial space [66]. Both superoxide dismutases SOD (Cu-Zn) (6 contigs) and SOD (Mn) (2 contigs) are identified in tardigrades (Table 3).

Glutathione transferases (GSTs) constitute a protein superfamily that is involved in cellular detoxification [67]. Furthermore GSTs have cellular physiology roles such as regulators of cellular pathways of stress response and housekeeping roles in the binding and transport of specific ligands [68]. The consequence of this diversity in role is the expression of multiple forms of GST in an organism as it is also the case in tardigrades. GSTs are divided into at least four major groups, namely cytosolic GSTs, mitochondrial GSTs, microsomal GSTs and bacterial fosfomycin-resistance proteins [69]. Mammalian cytosolic GSTs were classified into the Alpha, Mu and Pi classes [70]. Whereas several enzymes were recognized as belonging to the Alphas and Mu classes, Pi class contains only one protein. Different GST isoforms classified in diverse classes such as sigma, zeta, beta have been found also in other species like fungi, plants, helminthes and insects [70]. The wide taxonomic distribution leads to confirm the fundamental role of GSTs in protection against xenobiotics and endobiotics. We have found 27 different contigs that belong to sigma, mu and zeta protein families of GST superfamily.

Peroxiredoxins identified first in yeast [71] are conserved, abundant, thioredoxin peroxidase enzymes containing one or two conserved cysteine residues that protect lipids, enzymes, and DNA against reactive oxygen species. An important aspect of desiccation tolerance is protection against free radicals [72, 73]. Notably, the expression of 1-cysteine (1-Cys) peroxiredoxin family of antioxidants is reported in *Arabidopsis thaliana* and is shown to be related to dormancy [74]. Different isoforms of peroxiredoxins (8 contigs) are included in our result. Mainly Peroxiredoxins

and diverse other proteins like catalase, peroxidase, thioredoxin reductase and glutamate cysteine ligase are described to be involved in response to oxidative stress (Table 3).

**Table 3: Identified proteins with antioxidant activity in *M. tardigradum*.**

Protein annotation	emPAI		
	EES	AS	TS
<b>GST superfamily</b>			
contig22559:1:111:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-05	10.36	20.7	12.73
contig21746:1:589:2 similar to Glutathione S-transferase 1 (P46436) Evalue: 6e-32	6.63	19.22	21.28
contig11153:105:404:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-22	0.68	16.9	9.43
contig28284:1:272:3 similar to Glutathione S-transferase (P30116) Evalue: 2e-10	2.83	16.67	9.79
contig28262:1:137:2 similar to Glutathione S-transferase Mu 2 (P08010) Evalue: 2e-04	2.45	15.71	23
contig26098:1:627:1 similar to Glutathione S-transferase omega-1 (P78417) Evalue: 6e-39	6.53	9.78	11.18
contig27967:1:110:3 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-04	2.37	6.02	2.37
contig28200:1:335:1 similar to Glutathione S-transferase Mu 1 (Q9N0V4) Evalue: 4e-19	3.67	5.42	2.22
contig23837:230:385:2 similar to Probable glutathione S-transferase 7 (P91253) Evalue: 7e-08	0	2.71	3.6
contig11431:61:646:1 similar to Glutathione S-transferase omega-1 (P78417) Evalue: 4e-24	0.32	2.7	1.02
contig27884:1:341:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-10	2.06	2.6	1.03
contig17497:1:693:1 similar to Glutathione peroxidase (Q00277) Evalue: 5e-45	1.76	2.46	2.33
contig19542:93:722:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 1e-31	0.69	2.03	1.52
contig18624:208:876:1 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-34	0	1.95	1.3
contig27010:1:263:3 similar to Glutathione S-transferase class-mu 26 kDa isozyme 7 (P31671) Evalue: 2e-08	0	1.42	1.42
contig24978:1:466:2 similar to Glutathione S-transferase (O18598) Evalue: 4e-13	0	1.17	1.35
contig03609:1:822:1 similar to Glutathione S-transferase theta-1 (P20135) Evalue: 2e-28	0.6	1.13	0.82
contig22893:435:602:3 similar to Glutathione S-transferase (P46428) Evalue: 6e-07	0	1.08	1.08
contig05084:1:468:1 similar to Glutathione S-transferase (O18598) Evalue: 2e-11	0	0.97	0
contig25826:1:785:3 similar to Glutathione S-transferase omega-1 (Q9N1F5) Evalue: 2e-29	0	0.88	0.52
contig19224:1:520:3 similar to Glutathione S-transferase 2 (P20136) Evalue: 4e-30	0	0.83	0.57
contig18536:108:511:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-25	0	0.79	1.65
contig04771:2479:3345:1 similar to Glutathione S-transferase (P46419) Evalue: 1e-31	0.55	0.74	1.1
contig19966:1:524:3 similar to Microsomal glutathione S-transferase 2 (Q2KJG4) Evalue: 2e-17	3.03	0.51	1
contig21268:161:805:2 similar to Glutathione S-transferase 1 (P46434) Evalue: 1e-23	0	0.47	0.47
contig11340:1490:2110:2 similar to Glutathione S-transferase 1 (P46436) Evalue: 1e-30	0	0.31	0.49
contig25262:1:948:1 similar to Glutathione S-transferase (P46428) Evalue: 1e-19	0	0	0.18
Total emPAI	44.53	135.17	113.45
<b>Peroxiredoxins</b>			
contig21646:1:212:3 similar to Peroxiredoxin-2 (Q8K3U7) Evalue: 7e-29	3.63	7.06	3.92
contig00858:452:928:2 similar to Peroxiredoxin-6 (Q5ZJF4) Evalue: 3e-45	2.26	6.96	7.82
contig24044:157:536:1 similar to Peroxiredoxin-2 (Q2PFZ3) Evalue: 2e-33	4.02	3.19	4.62
contig23916:1:215:3 similar to Peroxiredoxin-2 (Q8K3U7) Evalue: 7e-30	0	3.12	3.12
contig24578:1:638:1 similar to Peroxiredoxin-4 (O08807) Evalue: 8e-51	2.32	2.82	2.25
contig20474:30:605:3 similar to Peroxiredoxin-5, mitochondrial (P30044) Evalue: 2e-48	0.9	1.15	0.72
contig17026:72:905:3 similar to Peroxiredoxin-2 (Q2PFZ3) Evalue: 5e-75	0.59	1.02	1.2
contig18110:116:595:2 similar to Peroxiredoxin-5, mitochondrial (Q9GLW9) Evalue: 2e-44	0.42	0	0
Total emPAI	14.14	25.32	23.65
<b>Superoxide dismutases</b>			
contig17128:1:666:1 similar to Superoxide dismutase [Cu-Zn] (Q8HXQ3) Evalue: 5e-52	10.94	7.3	5.72
contig06938:1:111:1 similar to Superoxide dismutase [Cu-Zn] (O46412) Evalue: 2e-08	24.66	7.12	5.3
contig10844:1:307:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 7e-25	29.04	6.87	15.04
contig21632:1:563:1 similar to Superoxide dismutase [Mn], mitochondrial (P09671) Evalue: 3e-44	3.68	4.95	7.48
contig00081:1:487:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 1e-42	3.68	4.38	3.88
contig24565:1:222:1 similar to Superoxide dismutase [Mn], mitochondrial (P09671) Evalue: 3e-21	0	0.8	0
contig07268:293:706:2 similar to Superoxide dismutase [Cu-Zn] (Q8HXQ2) Evalue: 2e-07	3.15	0.44	0.44
contig27339:102:582:3 similar to Superoxide dismutase [Cu-Zn], chloroplastic (P07505) Evalue: 4e-22	0	0	0.83
Total emPAI	75.15	31.86	38.69
<b>Others</b>			
contig01298:1:2065:3 similar to Catalase HPII (P21179) Evalue: 0.0	7.24	13.23	11.24
contig28039:246:1925:3 similar to Thioredoxin reductase 1, cytoplasmic (Q16881) Evalue: 1e-165	0.34	1.08	0.73

The comparison of the total emPAI of protein families with antioxidant activity listed in Table 3 shows that peroxiredoxins and in particular GSTs are highly regulated in adults compared to EES, which is probably due to the exposition to higher amounts of endobiotics and xenobiotics. Eggs are laid inside the old cuticle and remain there during the embryonic development. Therefore embryos are not directly attacked by xenobiotics. In contrast SODs are up-regulated in EES compared to adults. The studies on development of mouse embryos *in vitro* have shown that thioredoxin and SODs promote the in-vitro development of mouse embryos fertilized *in vitro* [75]. This suggests that protection of embryos from oxidative stress is a prerequisite for their development *in vitro*. We assume that the up-regulation of SODs in EES is related to their important roles in development. Comparing active to tun state we observed up-regulation of GSTs and peroxiredoxins in active state and in contrast up-regulation of SODs in tun state.

### Aquaporins

The result of protein analysis includes 350 transmembrane proteins, from which 65 are involved in transmembrane transport. 17 proteins are identified with channel transporter activity, most of which belong to ligand-gated ionic channel family (acetylcholine receptors proteins). One group of channel proteins that plays an important role in “desiccation-avoidance strategy” is the aquaporin (AQP) protein family. AQPs are passive transport channels for water, which means they permit water to move in the direction of an osmotic gradient. Kikawada et al. [74] analyzed two AQPs of *Polypedilum vanderplanki*, one of the largest anhydrobiotic animals. The result shows that one AQP controls the water homeostasis of fat body during normal conditions whereas the other one is involved in the removal of water in the desiccation process en route to anhydrobiosis. Different aquaporin (AQP) proteins are identified in tardigrades: AQP 3, AQP 4, AQP 9 (2 contigs), AQP 10 (2 contigs), AQP Z2 (Table 4). The question whether identified AQPs are involved in anhydrobiosis by *M. tardigradum* needs to be answered using functional analysis.

**Table 4: Identified aquaporins in *M. tardigradum*.** Aquaporins identified in different states are listed and the expression level is compared using emPAI. One peptide identifications are indicated with asterisk.

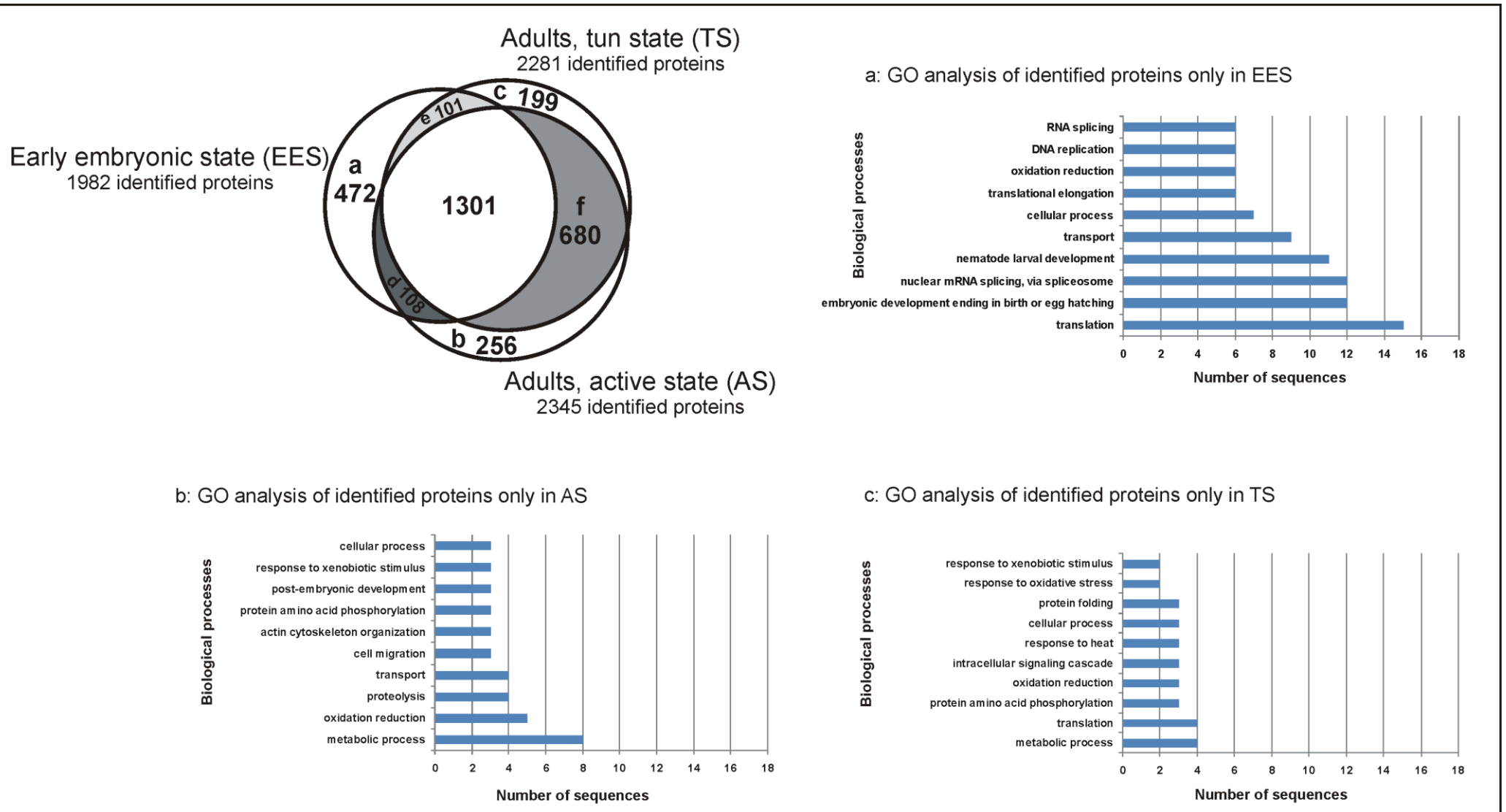
Protein annotation	emPAI		
	EES	AS	TS
contig03339:1:248:3 similar to Aquaporin-9 (O43315) Evalue: 1e-12*	0.39	/	/
contig23372:1:697:3 similar to Aquaporin Z 2 (Q8UJW4) Evalue: 7e-06*	/	/	0.13
contig17421:247:1275:1 similar to Aquaporin-4 (Q514F9) Evalue: 2e-36	15.82	5.52	6.56
contig04424:252:1300:3 similar to Aquaporin-10 (Q96PS8) Evalue: 4e-56	1.8	4.67	4.1
contig24282:1:693:1 similar to Aquaporin-10 (Q96PS8) Evalue: 3e-39	/	0.26	0.13
contig26144:1190:2191:2 similar to Aquaporin-9 (O43315) Evalue: 8e-43	/	0.18	/
contig01013:1:991:2 similar to Aquaporin-3 (Q08DE6) Evalue: 1e-39*	/	0.09	0.18

### 3.1.3 Comparative analysis of identified proteins in different states

The proteome analysis yielded 1982 unique proteins in early embryonic state (EES), 2345 unique proteins in adult tardigrades in active state (AS) and 2281 unique proteins in tun state (TS). A total of 1301 proteins are found in all three states as shown in the Venn diagram in Figure 6. 472 unique proteins are only identified in EES, 199 only in TS and 256 only in AS. To compare and demonstrate the main GO categories of biological process of proteins in single regions (Figure 6, Venn diagram, a-c) Blast2GO program was used. The highest abundant biological processes for each region are shown in Figure 6a-c.

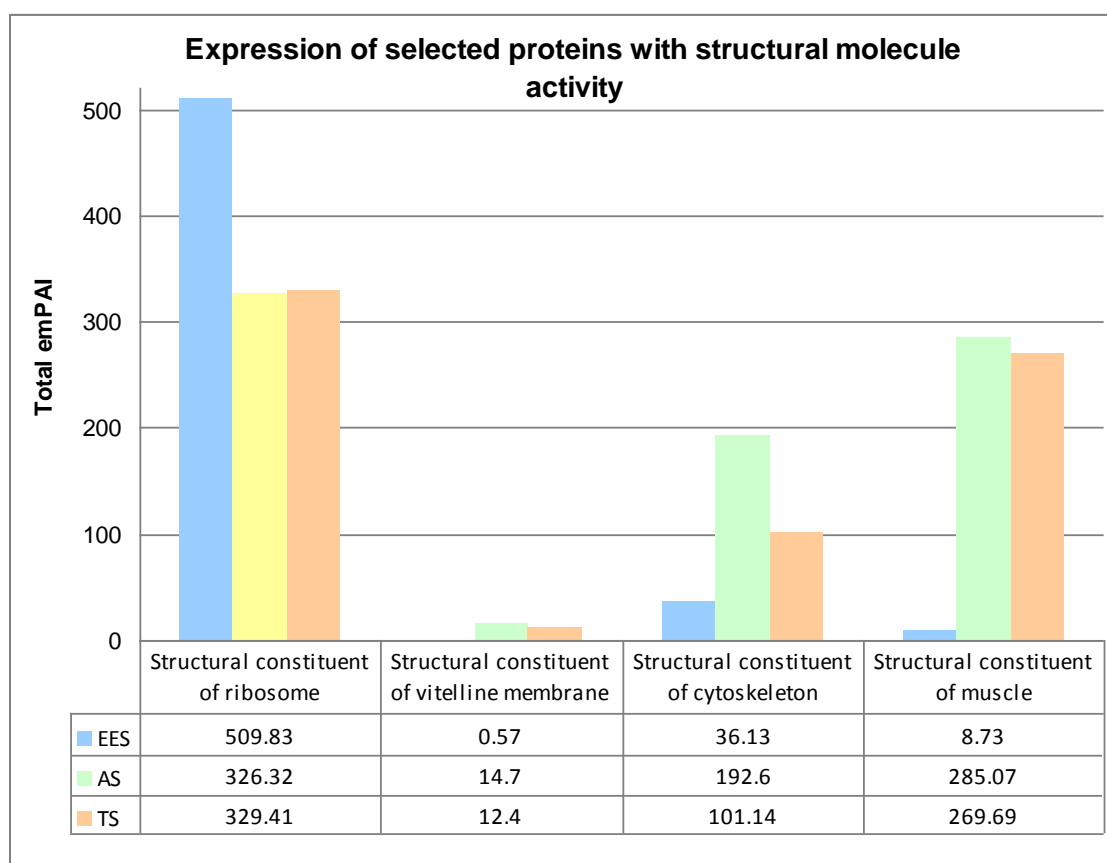
#### ***Proteins identified only in one state***

A total of 472 unique proteins were identified only in EES, from which 122 are without annotation. Among annotated proteins ribosomal proteins involved in translation represent the majority as shown in Figure 6a. Although ribosomal proteins are found in all states, there are diverse ribosomal proteins (32 unique proteins) that are only identified in EES. Comparison of calculated total emPAI of ribosomal proteins indicates their up-regulation in EES (Figure 7). The high regulation of ribosomal proteins in this state is due to the high need of protein synthesis with diverse functions including development en route to a mature organism. In contrast proteins contributing to the structural integrity of cytoskeletal, muscle and vitelline membrane are weakly expressed at this state. Since vitelline membrane is a portion of egg shell, we expect the expression of proteins in its formation only in mature animals, which is also reflected in semi-quantitative analysis (Figure 7). The second main protein category present in EES contains proteins involved in embryonic development, which is expected (Figure 6a).



**Figure 6: Comparative proteome analysis of identified proteins in different states.** The Venn diagram illustrates the number of protein identifications in EES, AS and TS. A total of 1301 unique proteins are found in all three states. Whereas 472 (a) unique proteins are found only in EES, we identified 680 (f) unique proteins only in adult tardigrades. Proteins of single (a, b, c) regions are analyzed using Blast2GO program to demonstrate the involved biological processes.

Specific proteins like protein members of piwi family are identified only in the EES. Piwi like proteins are developmental proteins that play a central role during gametogenesis. Proteins involved in iron homeostasis like soma ferritin are found in EES. In general four contigs annotated as proteins belonging to ferritin family are found (Supplementary Table 2), two of which are identified in all three states, one in EES and TS and another one only in EES. These proteins in particular the soma ferritin protein that is found only in EES are significantly up-regulated in EES as it is shown by comparing the emPAI. As described before two members of heat shock protein family are identified only in EES: the small heat shock protein C4 involved in stress response and 10 kDa heat shock protein belonging to the GroES chaperonin family involved in protein folding. In addition the small heat shock protein major egg antigen (p40) is included in the results, which is extremely up-regulated in EES. Similarly studies on the crustacean *Artemia franciscana*, which forms cysts in response to adverse conditions, show the presence of large amounts of artemin (a ferritin homologue) and a small heat shock protein (Hsp26) in the cyst [31, 34]. In studies on artemia it has been shown that the small heat shock protein and artemin are associated with anhydrobiosis. Since we found p40 and soma ferritin both up-regulated in EES and not in anhydrobiotic state, we assume that these proteins are involved in development and hence are specific markers for the EES. However, the role of p40 and ferritin in anhydrobiotic tardigrades has to be investigated.



**Figure 7: Analyzing the expression of selected proteins with structural molecule activity (defined by Gene Ontology).** Semi-quantitative analysis of proteins contributing to the structural integrity of ribosome, cytoskeletal, muscle and vitelline membrane structure shows the extreme up-regulation of ribosomal proteins in EES. In contrast proteins involved in cytoskeletal-, muscle- and vitellin membrane structure are not highly expressed at this state comparing to adults.

A total of 256 unique proteins are found only in AS, from which 71 proteins are without annotation. The two most abundant proteins (contig10378:1406:1499:2, contig26294:112:325:1) are without annotation and DomainSweep analysis delivered no specific protein domains. Dixin, a developmental protein involved in Wnt signalling pathway is the third highest abundant protein. Wnts are a large family of cysteine-rich secreted glycoproteins that controls development in organisms ranging from nematodes to mammals. The Blast2GO analysis of annotated proteins (Figure 6b) delivered metabolic process, oxidation reduction and proteolysis as first main categories, which are the main important processes for a living organism.

We identified 199 unique proteins in TS, from which 58 are without annotation. Two proteins without annotation (contig00440:1:127:2, contig24440:1:106:2) followed by myosin heavy chain (contig27485:90:265:3) are the most abundant proteins found only in TS. The result of Blast2GO analysis of annotated proteins is shown in Figure 6c. The first ten biological process categories include three categories involved in response to stimulus such as heat, oxidative stress and xenobiotic stimulus (Figure 6c). Only the last one is also present in AS (Figure 6b). This is due



to the permanent exposition to non-nutritional foreign chemical species (xenobiotics) in AS as well as in TS. Although activation of stress response was expected in TS, it seems there are other processes which are associated with anhydrobiosis. Proteins involved in intracellular signaling cascade and phosphorylation are present. Although protein amino acid phosphorylation as a biological process category is also present in the Blast2GO result of proteins identified only in AS (Figure 6b), the involved proteins are different compared to TS. Dual specificity mitogen-activated protein kinase (contig05524:314:1363:2), RAC serine/threonine-protein kinase (contig11071:1:467:3) and cell division cycle 2-like protein kinase 6 (contig17945:1:686:1), which are involved in phosphorylation have been identified only in TS. Also of major interest are proteins involved in intracellular signaling cascade: calcium-regulated heat stable protein 1 (contig02037:183:689:3), RAC serine/threonine-protein kinase (contig11071:1:467:3) and Drebrin-like protein (contig16604:102:1247:3). However, the role of these proteins in relation to desiccation tolerance has to be investigated. There are reports of observed changes in protein phosphorylation in plants which were exposed to water deficit, suggesting reversible phosphorylation as a regulator [76]. In particular mitogen-activated protein kinases (MAPKs) and other kinases belonging to the MAPK cascade have been identified in plants in response to dehydration, suggesting that the MAPK cascade is involved in stress signaling [77, 78]. In addition proteins like lipid storage droplets surface-binding protein, which is involved in lipid transport and is reported to be required for normal deposition of neutral lipids in the oocytes [79, 80], are identified only in TS. Lipids represent probably the only nutrient sources during anhydrobiosis and thus are essential for surviving. Heat shock protein 81-2 (Hsp90 family), hypoxia up-regulated protein 1 (Hsp70 family), and two members of DnaJ protein family as chaperones involved in stress response are identified only in tun state.

Proteins involved in metabolic processes are present in TS but reduced to half compared to AS, which is in accordance to the expectation since during anhydrobiosis (TS) a metabolic dormancy is described [16, 81]. Furthermore in contrast to proteins found only in AS translation is one of ten abundant processes in TS.

### ***Proteins overlapping in two states***

Whereas 680 proteins were identified only in adult tardigrades (active and tun), the number of proteins which are overlapping in EES and adults are significantly lower (108 between EES and AS and 101 between EES and TS) as expected (Venn diagram in Figure 6). Whereas cellular component organisation and transport are main processes in both EES and AS translation, development and biological regulation are abundant categories found in both EES and TS (data not shown). Proteins found only in AS and TS are mainly involved in cellular process, oxidation reduction, proteolysis and biological regulation.

## 3.2 Quantitative analysis of proteins in tardigrades in different states

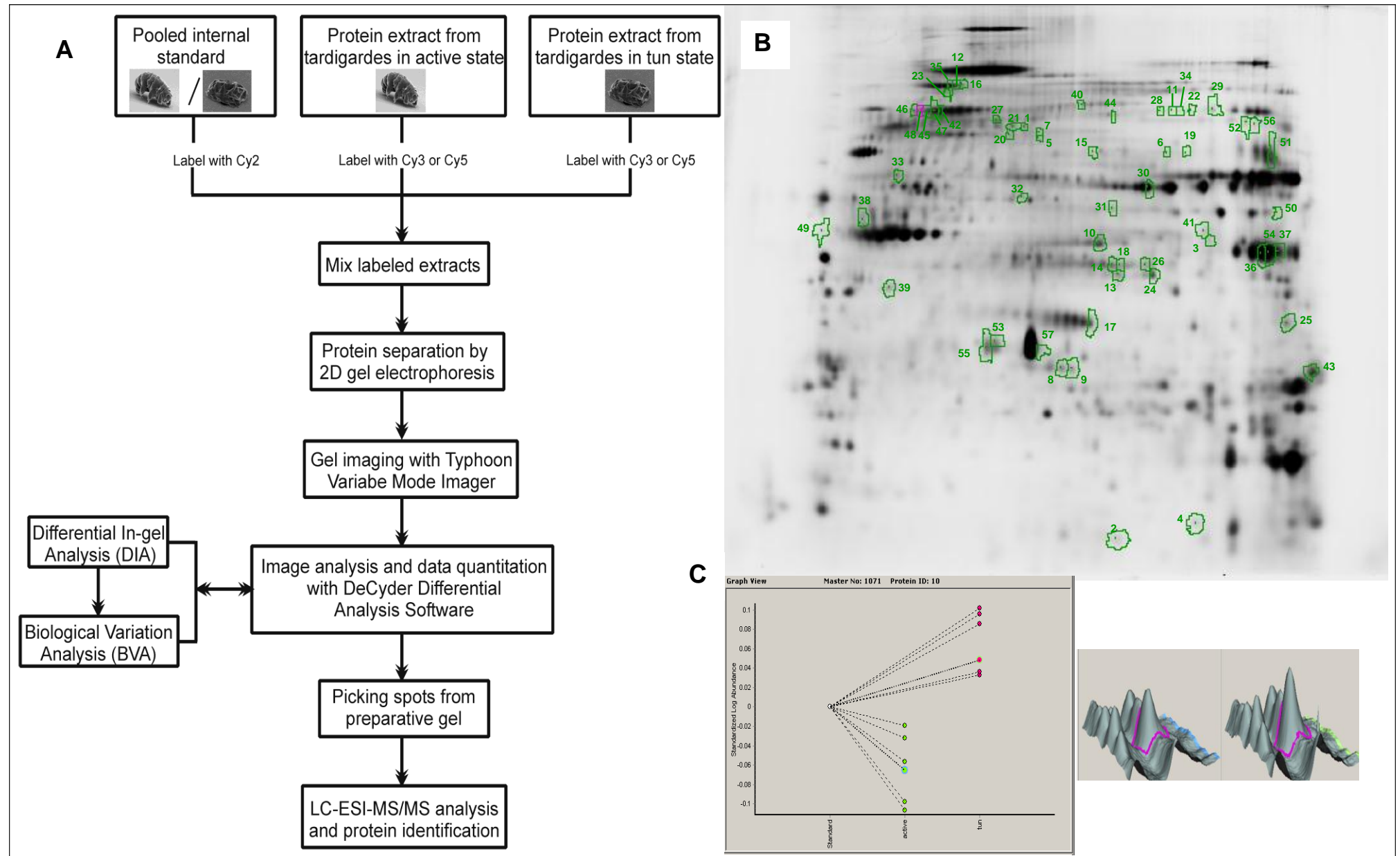
Not only the general profiling but also quantitative analyses of proteins that are differentially expressed between different states are of major interest. In particular this allows identifying proteins that play an important role in anhydrobiosis and helps better understanding of molecular processes. The quantitative analysis of tardigrades in different states was performed using two different methods: a) 2D DIGE (two-dimensional fluorescence difference gel electrophoresis), b) label-free technique based on emPAI to achieve a simple and fast quantification.

### 3.2.1 Two-dimensional fluorescence difference gel electrophoresis (2D DIGE)

To generate the protein expression profile of tardigrades in active state *versus* tun state, we performed two-dimensional fluorescence difference gel electrophoresis (2D DIGE). 2D DIGE is a quantitative method based on fluorescence labeling of proteins in different samples. Gel-to-gel variation is minimized by allowing the separation of two different samples which are labeled with different fluorescent dyes (Cy3, Cy5 respectively) on the same gel. In particular the minimal labeling approach allows using an internal standard (a pool of two different samples), which offers many advantages. The most important one is accurate quantification and spot statistics between the gels. Since the protein amount of one individual tardigrade is too low, we had to pool 1600 individuals for each state to include technical replications in our experiment. We pooled 1600 active tardigrades (pool 1) and 1600 anhydrobiotic tardigrades (pool 2). A total of six technical replicates and one preparative gel were prepared. Protein extracts of active and anhydrobiotic tardigrades were labeled alternately with Cy3 and Cy5 as shown in the experimental workflow in Figure 8. The internal standard was a pool that included an equal volume aliquot of both samples, which was labeled with Cy2 and separated on each of the gels. These three different labeled protein extracts were mixed in a ratio of 1:1:1 and separated by their isoelectric point in the first dimension and by molecular weight in the second dimension.

For image acquisition we used Typhoon Variable Mode Imager. We analyzed images with Decyder Differential Analysis Software, which allows differential in-gel analysis (DIA) and biological variation analysis (BVA). In total, up to 8000 different spots were detected on the gels as determined by the DeCyder Differential Analysis Software in DIA tool, from which 5000 were filtered out. Intergel matching was performed in BVA tool through the inclusion of the internal standard on each gel. Those protein spots that are localized at the same position in different gels were matched together and a total of 1470 protein spots were matched across all six gels.

To select the proteins of interest we set the parameter for protein filter as follows: Student's T-test  $<0,05$  and average ratio  $>1,25$  or  $<-1,25$ . Furthermore the selected protein spots had to be present in 75% of gel images, which means in 14 spot maps. In Figure 8C spot no 10 is shown in BVA module. The 3D diagram of spot 10 on two images corresponding to active and tun state is shown. The up-regulation of this protein in tun state is demonstrated by the height of the peak and furthermore by the graph view that shows the regulation of this protein among all replicates. The experimental design using an internal standard and 6 technical replicates resulted in a reliable and accurate statistical analysis. However, we could not detect regulations above or below 1.6fold. Positive average ratios indicate up-regulation in tun state and negative average ratios down-regulation as listed in Supplementary Table 3. A total of 57 spots passed the selected protein filter as shown in Figure 8B. All selected protein spots in the BVA tool except spot 2 and 4 could be localized on the preparative gel and picked for MS/MS analysis by electrospray ionization tandem mass spectrometry (ESI-MS/MS). We performed database search against the 454 tardigrade protein database. 42 protein spots could be identified, from which 23 protein spots contain more than one identified protein as listed in Supplementary Table 3. The highest up-regulated protein in tun state is identified as chaperone protein dnak that belong to the hsp70 family. Major egg antigen belonging to small heat shock proteins is also up-regulated in tun state and has been identified in two different spots as single hit (spots 3 and 6). The result of identified proteins from analyzed spots was difficult to interpret. One of the potential limitations of DIGE technology is that most spots on a given 2D gel contain more than one protein, making quantification difficult since it is not apparent which protein in the spot is regulated. Separation of the protein lysate by using a medium-pH-range IPG strip would help to reduce this problem. Since our biological material was limited, we had to choose the broad-pH-range IPG strips to screen nearly all proteins expressed in tardigrades. In addition, detecting and picking spots, which show high regulation but are present in low concentration is mostly not possible as shown for spot 2 and 4. Furthermore DIGE technology is subject to the restrictions imposed by the gel method. This includes the limited dynamic range, difficulty to separate hydrophobic proteins or proteins with high MW or pI on 2D gel. Therefore selecting and performing an alternative quantification method, which is suitable for our biological system was the next important step.



**Figure 8: Experimental workflow of DIGE experiment.** Samples were prepared and labeled with CyDye DIGE Fluor minimal dyes. The presence of an internal standard, a pool of tardigrade lysate in active and tun state in a ratio of 1:1, led to an accurate and reliable statistical result. Lysates of different states were labeled alternately with Cy5 or Cy3. All labeled extracts were mixed and separated performing 2D gel electrophoresis. The images were acquired with a Typhoon scanner. Analysis of protein spots using Decyder Differential Analysis Software resulted in localization of regulated proteins (B, indicated in green), which were picked from a preparative 2D gel and analyzed by LC-ESI-MS/MS. C) 3D diagram and graph view of spot 10 achieved in Decyder Software (BVA tool).

### 3.2.2 Semi-quantitative analysis using a label-free approach based on Exponentially Modified Protein Abundance Index (emPAI)

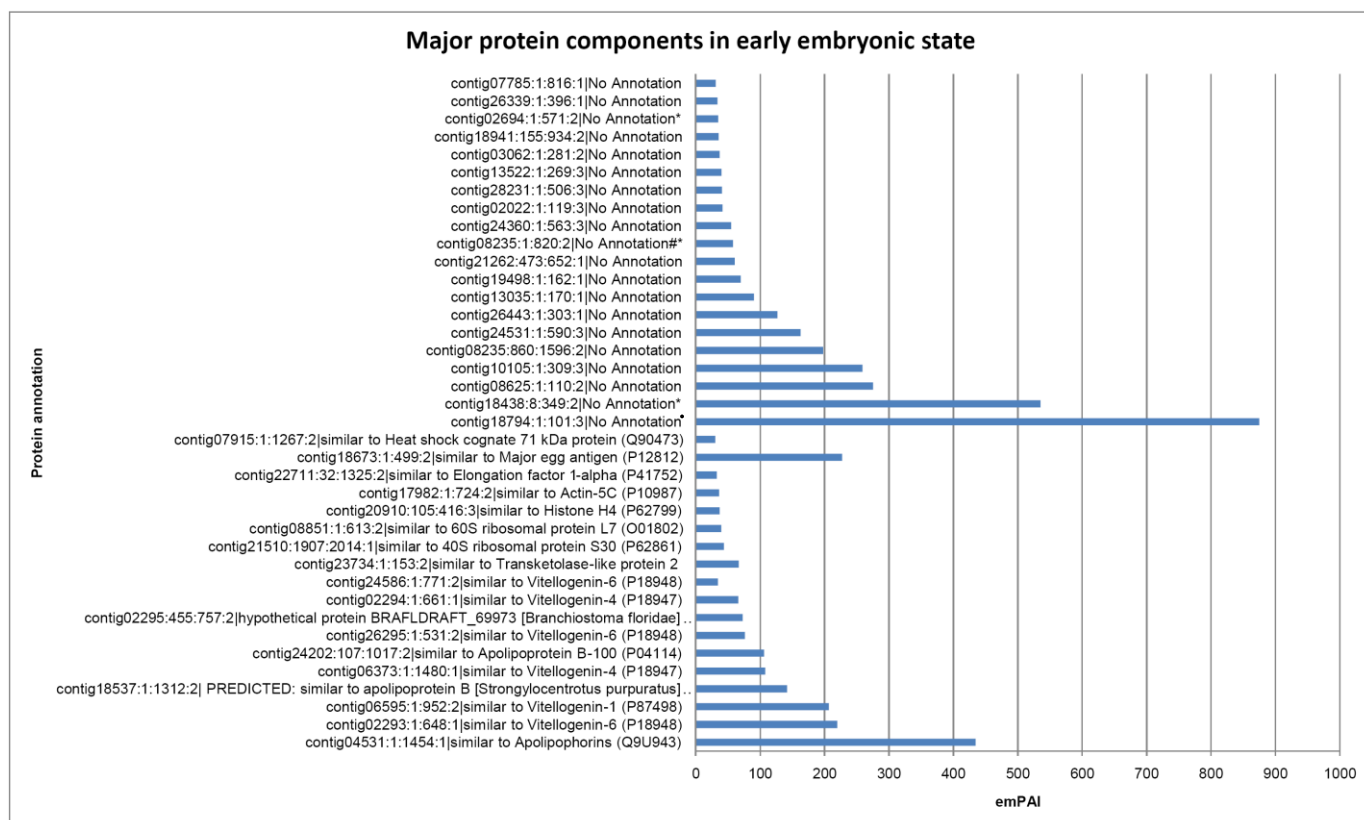
In the present work a semi-quantitative analysis of proteins differentially expressed in different states was performed using label-free technique based on emPAI to achieve a simple and fast quantification. The emPAI can be directly used for reporting approximate protein abundance in a large-scale analysis as shown in different studies [49, 51-54]. In our study the emPAI was used to obtain an approximate estimate of relative protein concentration by grouping the proteins into minor and major components and furthermore for semi-quantitative analysis of single proteins, which are differentially expressed in different states. This method employs normalization by interpreting signals of molecules that do not change concentration from sample to sample.

#### Determination of major components in early embryonic state comparing to adult animals in active and tun state

To analyze the major components in each different state we selected protein hits which show an emPAI of >30.

##### *Early embryonic state (EES)*

By selecting a cut-off of emPAI of >30, we found 38 proteins as major components, from which 20 are without annotation (Figure 9). Among annotated proteins we found 10 protein members of large lipid transporter (LLTP) family such as apolipoproteins and vitellogenins. Lipid transport in animals is mediated by members of the LLTP superfamily, which are grouped into three major families: the apoB-like LLTPs, the vitellogenin-like LLTPs and the microsomal triglyceride transfer protein (MTP)-like LLTPs or MTPs [82]. In addition to lipid transport they have also been reported to play an important role in animal development [83], reproduction [84] and immunity [85] as well as aging and lifespan regulation [86]. Whereas apoB is present in vertebrates, apolipoprotein-II/I (apoLp-II/I) are known as insect apolipoproteins. ApoB-like LLTPs are represented in our results by following protein members: 3 contigs which show high homology to apolipoprotein B, apolipoprotein O and apolipoprotein (Table 5).



**Figure 9: Major components in early embryonic state.** To classify proteins as major components we selected a cut-off of emPAI >30. We found 38 proteins, from which 10 belong to large lipid transporter superfamily. Members of heat shock protein family, structural constituent of ribosome and cytoskeleton are also present. In addition, 20 proteins are without annotation. The contig description is indicated with asterisk, in case we found putative candidates by DomainSweep analysis. For one contig DomainSweep analysis delivered a significant candidate (indicated with #). Proteins are ordered by their biological function.

Accumulation of the glycolipophosphoprotein vitellogenin (VTG) in oocytes is described to be one of the key processes in ovarian maturation. VTG is the major precursor of the egg-yolk proteins, vitellins (Vn), which provide sources of nutrients during embryonic development in oviparous organisms [84, 87]. It has been reported that lower vertebrates possess multiple VTG genes and proteins [87] as has been shown for *Danio rerio* [88], *Xenopus laevis* [89], *Caenorhabditis elegans* [90], salmonoid fishes [91]. Similarly multiple VTG proteins are found in *M. tardigradum* (Table 5): VTG-1, VTG-2, VTG-4 (2 contigs) and VTG-6 (3 contigs). Contig02295:455:757:2 annotated as hypothetical protein BRAFLDRAFT\_69973 contains one conserved vitellogenin-N domain and therefore belongs probably to large lipid transfer superfamily. Whereas apoB-like LLTPs and vitellogenin-like LLTPs are present in high abundance in our samples MTP-like LLTPs are underrepresented and are found only in adult tardigrades.

We found only one protein belonging to structural constituent of cytoskeleton (actin-5C) as major protein component in EES (Figure 9). Cytoskeleton proteins seem to be not highly expressed at this stage as have been shown also earlier (Figure 7) by calculating the total emPAI.

**Table 5: Identified proteins associated with lipid transport, metabolism and storage.**

GO term (category, level)	Protein annotation	emPAI		
		EES	AS	TS
<b>Lipid transport (BP, 4)</b>	<b>Large lipid transporter protein superfamily</b>			
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	434.48	148.6	131.78
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	106.16	47.92	45.38
	contig18537:1:1312:2  PREDICTED: similar to apolipoprotein B [Strongylocentrotus purpuratus] (XP_800206.2) Evalue: 1e-07	141.61	65.75	55.45
	contig24531:1:590:3 PREDICTED: apolipoprotein B [Danio rerio] (XP_694827.3) Evalue: 2e-5	162.83	38.74	55.8
	contig26593:129:609:3 uncharacterized conserved protein [Glossina morsitans morsitans] (ADD18598.1) Evalue: 1e-12	1.08	1.76	0.98
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	206.56	107.89	97.94
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	25.45	33.27	30.89
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	107.82	60.6	56.55
	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	65.94	95.06	73.72
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	76.29	37.64	40.05
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	34.26	55.07	60.25
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	219.67	134.37	113.52
	contig02295:455:757:2 hypothetical protein BRAFLDRAFT_69973 [Branchiostoma floridae] (XP_002591410.1) Evalue: 8e-04	72.73	58.29	47.7
	contig26982:1:1617:2 similar to Microsomal triglyceride transfer protein large subunit (P55158) Evalue: 4e-31	/	1.88	1.93
	<b>LDLR family (LDL binding proteins)</b>			
	contig23325:1:1472:3 similar to Very low-density lipoprotein receptor (P35953) Evalue: 4e-24	1.15	0.12	0.12
	contig19083:167:1503:2 similar to Low-density lipoprotein receptor (P35951) Evalue: 7e-05	0.36	1.67	1.52
	contig04458:347:1819:2 similar to Low-density lipoprotein receptor-related protein 5 (O75197) Evalue: 1e-20	/	0.12	0.12
	contig25676:1:412:3 similar to Very low-density lipoprotein receptor (P98155) Evalue: 9e-21	/	0.42	0.63
	contig25250:813:1145:3 similar to Low-density lipoprotein receptor-related protein 2 (A2ARV4) Evalue: 2e-10	0.56	2.56	2.38
	<b>HDL binding proteins</b>			
	contig20907:1:1151:1 similar to Vigilin (Q8VDJ3) Evalue: 2e-73	0.72	2.75	2.05
	contig23870:1:1221:3 similar to Vigilin (Q8VDJ3) Evalue: 1e-76	0.49	1.85	1.38
	<b>perilipin family</b>			
	contig18066:109:664:1 similar to Lipid storage droplets surface-binding protein 2 (Q9VXY7) Evalue: 2e-08	/	/	1.46
	contig23310:1:1017:1 similar to Perilipin-2 (Q9TUM6) Evalue: 8e-15	1.52	0.29	0.29
<b>Others</b>				
contig18095:1:491:3 similar to Aspartate aminotransferase, mitochondrial (P08907) Evalue: 6e-64	1.54	3.36	2.77	
contig05263:1:701:3 similar to Phosphatidylinositol transfer protein alpha isoform (P48738) Evalue: 6e-71	0.77	2.56	0.98	
contig22720:1:938:3 similar to Glycolipid transfer protein domain-containing protein 1 (Q6DBQ8) Evalue: 7e-27	/	0.43	0.31	
contig18709:206:705:2 similar to Epididymal secretory protein E1 (P61918) Evalue: 2e-16	0.4	0.4	0.4	
Total emPAI	<b>1662.39</b>	<b>903.37</b>	<b>826.35</b>	

Two ribosomal proteins 60S ribosomal protein L7 and 40S ribosomal proteins S30 belong to the category of major protein components (Figure 9) and are involved in translation and in particular 60S ribosomal protein L7 is known to be involved in reproduction and embryonic development ending in birth or egg hatching.

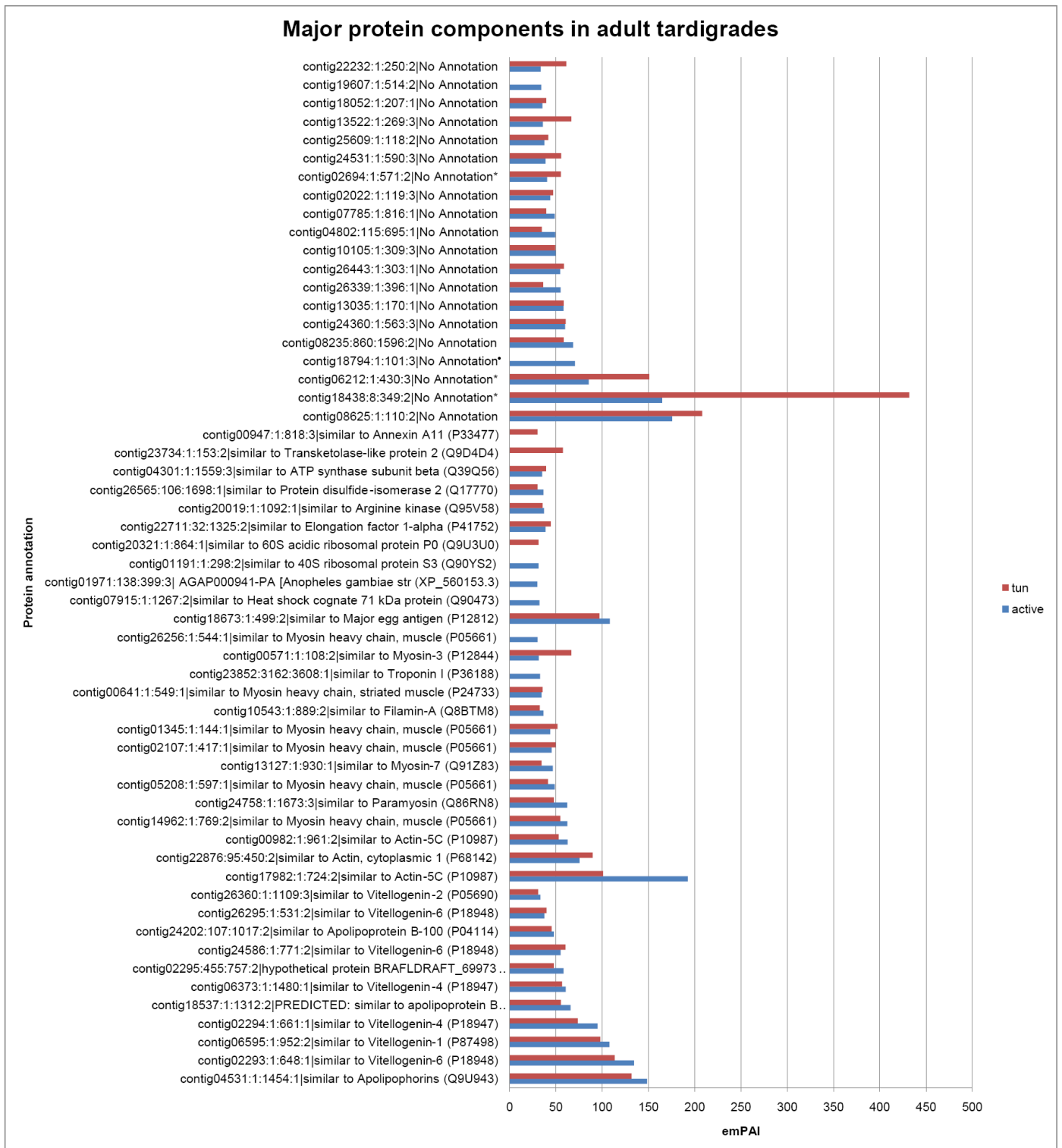
Two heat shock proteins, hcs71 and the small heat shock protein major egg antigen are present also in the major protein components category of EES. Comparing to hcs71 major egg antigen

is significantly up-regulated. Proteins without annotation are indicated with asterisk, in case we found putative candidates in DomainSweep results. For one contig (08235:1:820:2) DomainSweep analysis delivered a significant candidate (indicated with #), namely whey acidic protein (WAP) 4-disulfide core. This protein has a peptidase inhibitor activity. The highest abundant protein among proteins without annotation (contig18794:1:101:3) seems to belong to structural constituent of ribosome. Blast search against NCBI nr delivered ribosomal protein L4 (*Danio rerio*) however with an insufficient e-value. DomainSweep analysis of this protein resulted in ribosomal protein L4/L1e as putative candidate. Contig18438:8:349:2 is the second highest abundant protein among not annotated proteins. The result of Blast search against NCBI nr is not significant, but we could find a vWF (von Willebrand factor) conserved protein domain, which was confirmed by DomainSweep analysis as putative candidate. Protein members belonging to the apoB-like LLTPs and the vitellogenin-like LLTPs have two conserved protein domains: vitellogenin domain and vWF. Since we do not have any further information and only the presence of vWF domain is not specific for vitellogenin- and apoB-like LLTPs, we can not annotate this contig significantly.

#### *Adult tardigrades in active state (AS) and tun state (TS)*

By selecting a cut-off of emPAI >30, we found 53 proteins as major components in adult tardigrades in AS and 49 in TS (Figure 10). Comparing the annotated proteins in AS and TS we found the same two major functional groups, protein members of structural constituent of cytoskeleton/muscle and protein members of large lipid transporter family. The same protein members of large lipid transporter superfamily are present in AS as well as in TS. The following vitellogenin proteins are included: VTG-1, VTG-2, VTG-4 (2 different contig), VTG-6 (3 different contigs). The early embryonic state contains all these vitellogenins except for vitellogenin-2. Interestingly, vitellogenin-2 is described to be involved in biological process of determination of adult lifespan, which means the control of viability and duration in the adult phase of the life-cycle. Actin 5-C (2 contigs), cytoplasmic actin, filamin-A belong to the structural constituent of cytoskeleton. Myosin heavy chain, paramyosin, myosin-7, myosin-3, troponin I are muscle proteins and except troponin I have all motor activity function. We found one isoform of myosin heavy chain, troponin I, two protein members of heat shock protein family (Hsc 71 and AGAP000941-PA) and 40S ribosomal protein S3 as major components in AS, and annexin A11, transketolase-like protein 2 and 60S acidic ribosomal protein P0 as member of major component group in TS. Contig01971:138:399:3 is annotated as AGAP000941-PA from *Anopheles gambiae*, which shows high homology to small heat shock proteins. Among proteins without annotation, there are 18 proteins present in both AS and TS. In particular contig18438:8:349:2 (contains vWF conserved domain), which is the second highest abundant protein in EES (see previous chapter) is highly expressed in TS compared to AS.





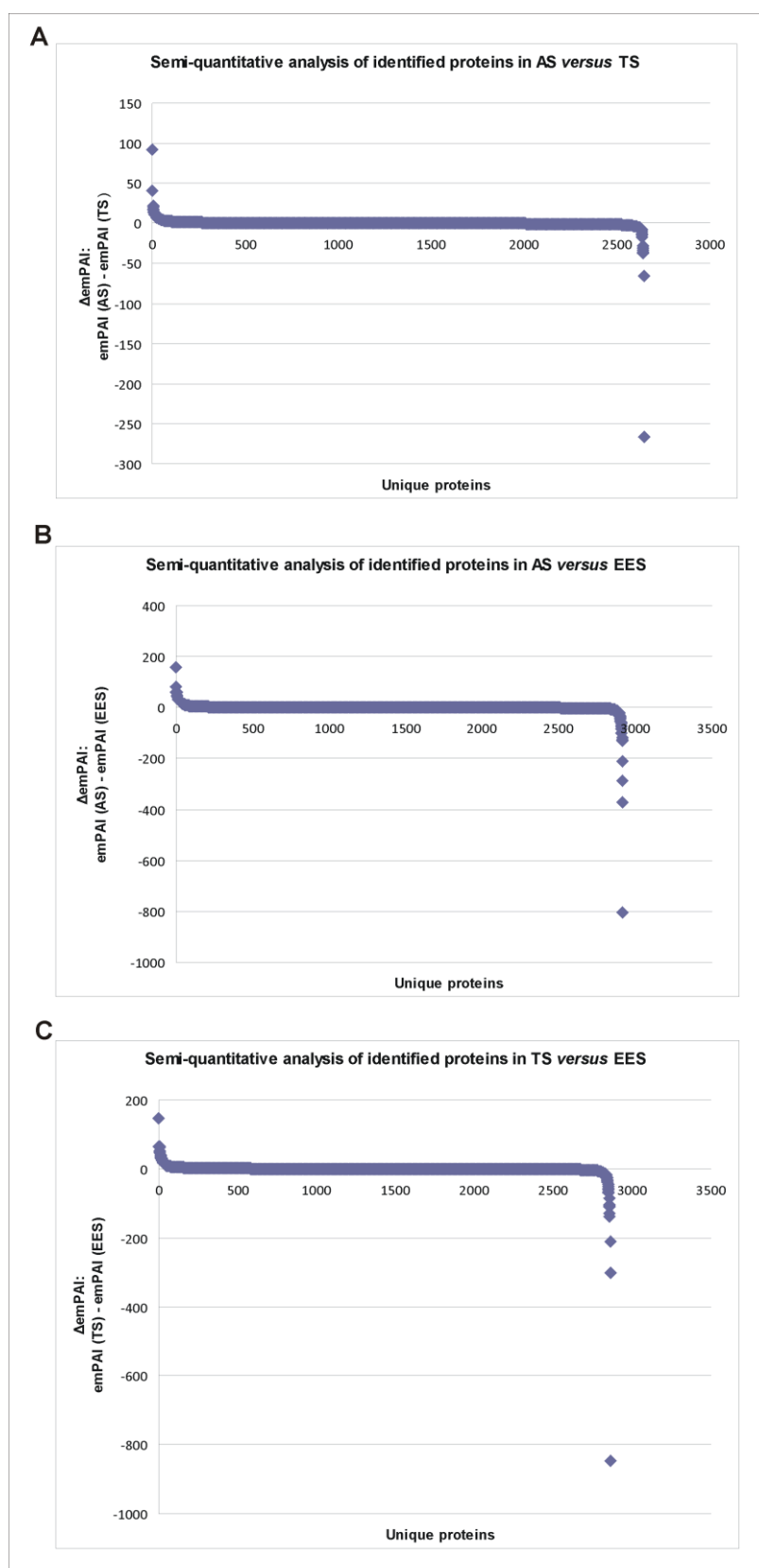
**Figure 10: Major components in adult tardigrades in active and tun state.** To classify proteins as major components we selected a cut-off of emPAI >30. We found 53 proteins as major components in adult tardigrades in AS and 49 in TS. Comparing the annotated proteins in AS and TS we found the same two major functional groups, protein members of structural constituent of cytoskeleton/muscle and protein members of large lipid transporter family. The contig description is indicated with asterisk, in case we found putative candidates in DomainSweep result. Proteins are ordered by their biological function.

## Semi-quantitative analysis of identified proteins in different states

In addition to grouping proteins in major and minor components the semi-quantitative analysis based on emPAI can be used for the relative quantification of single proteins. The semi-quantitative analysis between two different states was performed by calculating differences between emPAI for single proteins in each state. We have defined a cut-off of  $>10$  and  $<-10$  for  $\Delta\text{emPAI}$ . Proteins which show differences in  $\Delta\text{emPAI}$  over the cut-off are defined as differentially regulated in different states. The selected label-free method employs normalization by interpreting signals of molecules that do not change concentration from sample to sample. Whereas 98.87% of proteins identified in active and tun states are similar in regulation, we found up to 96% of proteins in EES and adults similar in regulation, which is according to our expectation.

### *Active state versus tun state*

The comparison of active *versus* tun state yielded 2614 contigs (98.87%) which are similar in regulation (Figure 11A). 30 contigs (1.13%) delivered differences in  $\Delta\text{emPAI}$ , which indicate differential regulation in proteins. These proteins are listed in Table 6 with the corresponding  $\Delta\text{emPAI}$  that allows a direct comparison of differentially regulated proteins in both states. 17 proteins are up-regulated in active state, from which 4 contigs could not be annotated significantly. Among the proteins with annotation we found proteins that belong to cytoskeleton (actin-5C), muscle protein family (paramyosin, myosin-7, troponin I), large lipid transfer superfamily (vitellogenin-4, vitellogenin-6, apolipoporphins, apolipoprotein B, hypothetical protein BRAFLDRAFT\_69973) and heat shock protein family (two sHsps and Hsc71). There are 13 up-regulated proteins in tun state, from which 8 contigs are without significant annotation. We found contig18438:8:349:2 (contains vWF conserved domain) as extremely high regulated in tun state, which was described in earlier chapters (determination of major components). Blast search against NCBI nr for the second highly expressed protein (contig06212:1:430:3) in tun state delivered hypothetical protein Phum\_PHUM355660 however with an insufficient e-value. DomainSweep analysis delivered one putative hit (IPR007282 NOT2/NOT3/NOT5), which is described to be involved in regulation of transcription. Among annotated proteins myosin-3, transketolase-like protein, apolipoprotein B, cytoplasmic actin and 40S ribosomal protein S3 are the other up-regulated proteins in tun state. Validation of the present result using other quantification methods is one of our future steps. Furthermore proteins that could not be annotated by homology search are of major interest and have to be functionally analyzed in regard to their association with anhydrobiosis.



**Figure 11: Determination of differentially expressed proteins by calculating  $\Delta\text{emPAI}$ .** The semi-quantitative analysis between two different states was performed by calculating the differences between emPAI for single proteins in each state. We have defined a cut-off of  $>10$  and  $<-10$  for  $\Delta\text{emPAI}$  to assign proteins as regulated. A) The comparison of active *versus* tun state yielded 2614 contigs (98.87%) which are similar in regulation. B) The comparison of active state *versus* early embryonic state yielded 2807 contigs (96.23%) which are similar in regulation. C) The comparison of tun state *versus* early embryonic state yielded 2759 contigs (96.40%) which are similar in regulation.

**Table 6: Semi-quantitative analysis of differentially expressed proteins in AS and TS.** Proteins without annotation are indicated with asterisk, in case we found putative candidates by DomainSweep analysis. The protein domain with IPR number is included in brackets. Under selected cut-off of >10 and <-10 for  $\Delta$ emPAI a total of 17 proteins are found to be up-regulated in AS. Cytoskeleton and muscle proteins (4 proteins), large lipid transporter superfamily (5 proteins) and heat shock proteins (3 proteins) are the major groups. 13 proteins are up-regulated in TS, from which 7 proteins could not be annotated by homology search.

no.	State/Slice no.	Accession no./Protein description	Protein score	Peptide matches (unique peptides)	Protein coverage	emPAI (AS)	emPAI (TS)	emPAI (AS-TS)
1	A13,11-15, 17-19	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	1143	182 (16)	76.2	192.6	101.14	91.46
2	A12, 12-19	contig18794:1:101:3 No Annotation*[IPR002136 Ribosomal protein L4/L1e]	192	6 (5)	97	70.62	29.55	41.07
3	A7, 5-19	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	774	59 (12)	73.2	95.06	73.72	21.34
4	A7, 4-19	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	810	72 (10)	51.4	134.37	113.52	20.85
5	A17, 14-18	contig26339:1:396:1 No Annotation	621	43 (10)	83.2	55.04	36.42	18.62
6	A14	contig18068:1:139:2 No Annotation	152	9 (4)	64.4	24.53	7.67	16.86
7	A5, 2-20	contig04531:1:1454:1 similar to Apolipoproteins (Q9U943) Evalue: 7e-11	2337	168 (33)	68.6	148.6	131.78	16.82
8	A11, 5, 7, 9-25	contig04802:1:115:695:1 No Annotation	709	86 (14)	73.1	49.23	34.66	14.57
9	A7, 4-19	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	2744	111 (42)	62.4	62.28	47.74	14.54
10	A13, 11-16	contig01971:1:38:399:3  AGAP000941-PA [Anopheles gambiae str. PEST] (XP_560153.3) Evalue: 8e-05	425	17 (7)	58.6	30.05	16.78	13.27
11	A7, 2-18	contig13127:1:930:1 similar to Myosin-7 (Q91Z83) Evalue: 1e-116	1071	33 (16)	64.5	46.57	34.4	12.17
12	A11, 4-19	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	793	52 (12)	75.3	108.34	97.24	11.1
13	A9, 8-18	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	1653	79 (26)	64.2	32.48	21.57	10.91
14	A16, 14-20	contig23852:3:162:3608:1 similar to Troponin I (P36188) Evalue: 2e-23	595	35 (9)	43.2	32.94	22.06	10.88
15	A7, 4-19	contig02295:455:757:2 hypothetical protein BRAFLDRAFT_69973 [Branchiostoma floridae] (XP_002591410.1) Evalue: 8e-04	290	32 (5)	52.5	58.29	47.7	10.59
16	A5, 2-19	contig18537:1:1312:2  PREDICTED: similar to apolipoprotein B [Strongylocentrotus purpuratus] (XP_800206.2) Evalue: 1e-07	1373	99 (21)	51.5	65.75	55.45	10.3
17	A16, 16-18	contig26452:1:179:2 similar to Elongation factor 1-beta~ (P29522) Evalue: 2e-21	315	9 (5)	84.7	27.26	17.24	10.02
18	A17, 16-20	contig07695:1:240:1 No Annotation	352	13 (6)	65	16.38	26.99	-10.61
19	A16, 4, 5, 14-17, 20	contig26860:1:33:289:1 similar to 40S ribosomal protein S3 (Q90YS2) Evalue: 3e-13	226	10 (4)	57.7	9.77	22.28	-12.51
20	A13, 4-8, 10-20	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	461	87 (7)	74.6	75.63	89.85	-14.22
21	A11, 6-20	contig02694:1:571:2 No Annotation*[IPR002181 Fibrinogen, IPR000463 Cytosolic fatty-acid binding]	973	96 (16)	72	40.57	55.6	-15.03
22	A5, 3-19	contig24531:1:590:3  PREDICTED: apolipoprotein B [Danio rerio] (XP_694827.3) Evalue: 2e-05	888	30 (11)	77.6	38.74	55.8	-17.06
23	A17, 16-19	contig22232:1:250:2 No Annotation	427	18 (6)	79.5	33.65	61.15	-27.5
24	A9, 8-16, 18, 19	contig23734:1:153:2 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 6e-16	246	9 (4)	78	29.43	57.59	-28.16
25	A7, 4-18	contig13522:1:269:3 No Annotation	267	11 (6)	50.6	35.97	66.72	-30.75
26	A7, 4-20	contig08625:1:110:2 No Annotation	264	17 (3)	69.4	175.51	208.2	-32.69
27	A12, 4, 6, 10, 14, 15	contig00571:1:108:2 similar to Myosin-3 (P12844) Evalue: 4e-08	144	5 (4)	71.4	31.51	66.84	-35.33
28	A17, 18	contig27733:1:294:1 No Annotation	464	20 (8)	71.4	29.46	66.66	-37.2
29	A15	contig06212:1:430:3 No Annotation*[IPR007282 NOT2/NOT3/NOT5]	618	49 (7)	93	85.41	150.89	-65.48
30	A6, 4-19	contig18438:8:349:2 No Annotation*[IPR001846 von Willebrand factor, type D]	484	42 (6)	61.1	164.93	431.91	-266.98

*Adults versus tardigrades in early embryonic state*

The comparison of active state *versus* early embryonic state yielded 2807 contigs (96.23%) which are similar in regulation (Figure 11B). 110 contigs (3.77 %) delivered differences in  $\Delta\text{emPAI}$ , which indicate differential regulation in proteins (Table 7). 62 proteins are up-regulated in active state, from which 15 contigs are without annotation. The major protein groups, which are highly expressed in active state, belong to structural constituent of cytoskeleton and muscle (21 proteins), antioxidant proteins (5 proteins), transmembrane transporters (4 proteins). Actin is the first up-regulated proteins in active state.

We found 48 proteins up-regulated in early embryonic state, from which 17 are without annotation. As expected members of large lipid transporter superfamily (8 proteins) are highly regulated in early embryonic state. Other protein families such as ribosomal proteins (7 proteins), antioxidant proteins (2 proteins) are present. Comparing the up-regulated antioxidant proteins we found that GSTs are up-regulated in active state while SODs are up-regulated in early embryonic state (see also chapter 3.1.2). The first two up-regulated proteins in early embryonic state are proteins without annotation. Especially contig18794:1:101:3, which is highly expressed in early embryonic state, seems to belong to structural constituent of ribosome (as described before). Contig18438:8:349:2 contains a vWF conserved domain, but it could not be annotated significantly. Among up-regulated proteins in EES we found furthermore soma ferritin and major egg antigen, which were discussed earlier (chapter 3.1.3) and developmental proteins such as muscle LIM protein 1.

The comparison of tun state *versus* early embryonic state yielded 2759 contigs (96.40%) which are similar in regulation (Figure 11C). 103 contigs (3.60 %) delivered differences in  $\Delta\text{emPAI}$ , which indicate differential regulation in proteins (result not included). We found 54 proteins (including 13 proteins without annotation) up-regulated in tun state and 49 proteins (including 19 proteins without annotation) up-regulated in early embryonic state. We found here the same regulated functional protein groups as described in the last section comparing active state and early embryonic state. The first high abundant protein in tun state is contig06212:1:430:3, which was also up-regulated in active state (second high abundant protein). In early embryonic state we found again contig18794:1:101:3 and contig18438:8:349:2 as highly abundant proteins, with the difference in their priorities; contig18794:1:101:3 is the highest abundant protein and contig18438:8:349:2 is in the 9. position. In particular contig18438:8:349:2 behaves similarly in its regulation in the early embryonic state and tun state compared to active state.

**Table 7: Semi-quantitative analysis of differentially expressed proteins in EES and AS.** Proteins without annotation are indicated with asterisk, in case we found putative candidates by DomainSweep analysis. The protein domain with IPR number is included in brackets. Under selected cut-off of >10 and <-10 for  $\Delta$ emPAI a total of 62 proteins are found to be up-regulated in AS from which 21 proteins are cytoskeleton and muscle proteins. Among proteins that are up-regulated in EES (48 proteins) protein members of large lipid transporter superfamily (9 proteins) and ribosomal proteins (7 proteins) build the majority.

no.	Accession no./Protein description	Protein score	Peptide matches (unique peptides)	Protein coverage	emPAI (AS)	emPAI (EES)	emPAI (AS-EES)
1	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	1143	182 (16)	76.2	192.6	36.13	156.47
2	contig06212:1:430:3 No Annotation*[IPR007282 NOT2/NOT3/NOT5]	618	49 (7)	93	85.41	3.97	81.44
3	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	2744	111 (42)	62.4	62.28	2.07	60.21
4	contig14962:1:769:2 similar to Myosin heavy chain, muscle (P05661) Evalue: 6e-58	1261	35 (21)	64.7	62.42	3.7	58.72
5	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	461	87 (7)	74.6	75.63	25.87	49.76
6	contig05208:1:597:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 3e-50	810	21 (12)	62.3	48.82	1.07	47.75
7	contig02107:1:417:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-35	526	16 (9)	57.6	45.53	0.42	45.11
8	contig01345:1:144:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 2e-08	233	4	54.2	43.96		43.96
9	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	1153	183 (16)	57.4	62.7	18.86	43.84
10	contig13127:1:930:1 similar to Myosin-7 (Q91Z83) Evalue: 1e-116	1071	33 (16)	64.5	46.57	3.24	43.33
11	contig10543:1:889:2 similar to Filamin-A (Q8BTM8) Evalue: 1e-72	1124	48 (18)	73.2	36.73	1.35	35.38
12	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	1592	85 (26)	73.3	37.1	1.76	35.34
13	contig25609:1:118:2 No Annotation	251	11 (4)	97.4	37.44	2.37	35.07
14	contig18052:1:207:1 No Annotation	380	14 (6)	82.6	35.33	0.86	34.47
15	contig00641:1:549:1 similar to Myosin heavy chain, striated muscle (P24733) Evalue: 3e-50	553	15 (10)	53	34.33		34.33
16	contig00571:1:108:2 similar to Myosin-3 (P12844) Evalue: 4e-08	144	5 (4)	71.4	31.51		31.51
17	contig26256:1:544:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-37	619	13 (7)	43.1	30.15	0.88	29.27
18	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	774	59 (12)	73.2	95.06	65.94	29.12
19	contig23852:3162:3608:1 similar to Troponin I (P36188) Evalue: 2e-23	595	35 (9)	43.2	32.94	4.97	27.97
20	contig00947:1:818:3 similar to Annexin A11 (P33477) Evalue: 6e-48	1301	52 (22)	78.6	29.14	1.44	27.7
21	contig04802:115:695:1 No Annotation	709	86 (14)	73.1	49.23	21.54	27.69
22	contig01971:138:399:3  AGAP000941-PA [Anopheles gambiae str. PEST] (XP_560153.3) Evalue: 8e-05	425	17 (7)	58.6	30.05	2.37	27.68
23	contig15494:1:257:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 8e-18	331	8 (4)	42.4	29.04	1.94	27.1
24	contig22232:1:250:2 No Annotation	427	18 (6)	79.5	33.65	7.68	25.97
25	contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	721	20 (9)	56.2	25.71	0.88	24.83
26	contig27733:1:294:1 No Annotation	464	20 (8)	71.4	29.46	5.72	23.74
27	contig18068:1:139:2 No Annotation	152	9 (4)	64.4	24.53	2.66	21.87
28	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	2450	138 (40)	70.4	36.48	14.61	21.87
29	contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	1992	120 (26)	75.1	34.93	13.17	21.76
30	contig26128:1:2657:2 similar to Myosin-3 (P12844) Evalue: 0.0	2180	67 (36)	49.3	22.39	0.72	21.67

no.	Accession no./Protein description	Protein score	Peptide matches (unique peptides)	Protein coverage	emPAI (AS)	emPAI (EES)	emPAI (AS-EES)
31	contig26339:1:396:1 No Annotation	621	43 (10)	83.2	55.04	33.54	21.5
32	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	725	60 (12)	55.5	55.07	34.26	20.81
33	contig01191:1:298:2 similar to 40S ribosomal protein S3 (Q90YS2) Evalue: 2e-32	343	20 (6)	81.6	31.11	11.11	20
34	contig22479:1:399:3 similar to Tropomyosin (Q95VA8) Evalue: 1e-38	560	20 (11)	58.3	19.98	1.04	18.94
35	contig07785:1:816:1 No Annotation	831	43 (15)	79.4	48.7	31.07	17.63
36	contig25519:1:245:2 similar to Fructose-bisphosphate aldolase, muscle type (P53445) Evalue: 3e-36	267	13 (5)	81.5	19.21	2.52	16.69
37	contig22765:1:560:3 similar to CD109 antigen (Q6YHK3) Evalue: 3e-16	422	12 (8)	40	17.92	1.54	16.38
38	contig11153:105:404:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-22	443	17 (9)	76	16.9	0.68	16.22
39	contig24401:1:658:2 similar to Malate dehydrogenase, mitochondrial (Q5NVR2) Evalue: 6e-85	995	36 (14)	79.8	25.04	9.1	15.94
40	contig03818:1:573:2 similar to Alpha-actinin, sarcomeric (P18091) Evalue: 9e-103	824	20 (12)	74.2	15.07		15.07
41	contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	1080	48 (17)	58.4	14.7	0.57	14.13
42	contig11420:186:337:3 similar to Actin (Q9UVX4) Evalue: 1e-25	99	10 (2)	54	19.32	5.26	14.06
43	contig28284:1:272:3 similar to Glutathione S-transferase (P30116) Evalue: 2e-10	361	15 (8)	76.4	16.67	2.83	13.84
44	contig28262:1:137:2 similar to Glutathione S-transferase Mu 2 (P08010) Evalue: 2e-04	120	9 (2)	71.1	15.71	2.45	13.26
45	contig13125:303:769:3 similar to Myosin heavy chain, muscle (P05661) Evalue: 2e-52	295	7 (6)	52.3	13.56	0.38	13.18
46	contig26155:1:131:3 similar to Voltage-dependent anion-selective channel protein 1 (Q60932) Evalue: 4e-09	115	8 (1)	42.9	20.41	7.25	13.16
47	contig05470:1:464:3 similar to Fructose-bisphosphate aldolase A (P05065) Evalue: 6e-54	528	17 (10)	68	16.75	3.69	13.06
48	contig21746:1:589:2 similar to Glutathione S-transferase 1 (P46436) Evalue: 6e-32	716	42 (11)	60	19.22	6.63	12.59
49	contig26452:1:179:2 similar to Elongation factor 1-beta~ (P29522) Evalue: 2e-21	315	9 (5)	84.7	27.26	14.85	12.41
50	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	1738	70 (28)	58.5	17.19	4.85	12.34
51	contig25313:1:118:2 No Annotation	144	4 (3)	57.9	12.33		12.33
52	contig09723:1:288:1 No Annotation	229	15 (2)	46.9	16.77	4.5	12.27
53	contig04440:420:1643:3 No Annotation	891	21 (16)	48.6	11.92		11.92
54	contig27035:1:393:1 similar to Carboxylesterase 2 (O00748) Evalue: 6e-26	486	18 (8)	77.1	12.41	0.51	11.9
55	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	1520	96 (23)	62.5	23.08	11.27	11.81
56	contig23401:118:845:1 similar to Voltage-dependent anion-selective channel protein 2 (P81004) Evalue: 2e-41	892	43 (16)	76.9	22.08	10.78	11.3
57	contig19230:752:868:2 No Annotation	149	6 (3)	61.5	10.76		10.76
58	contig26921:1:94:2 No Annotation	84	2 (1)	33.3	16.76	6.2	10.56
59	contig20024:1:210:1 similar to Heterochromatin-associated protein MENT (O73790) Evalue: 3e-07	164	8 (4)	58	10.73	0.22	10.51
60	contig00520:1:1212:1 No Annotation*[IPR006575 RWD]	1284	50 (24)	54.8	11.91	1.51	10.4
61	contig22559:1:111:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-05	194	12 (4)	81.1	20.7	10.36	10.34
62	contig23461:107:1792:2 similar to ATP synthase subunit alpha (A0LDA2) Evalue: 0.0	1774	76 (29)	51.5	16.86	6.84	10.02
63	contig17421:247:1275:1 similar to Aquaporin-4 (Q5I4F9) Evalue: 2e-36	271	11 (5)	15.2	5.52	15.82	-10.3
64	contig22152:1:228:1 similar to 60S ribosomal protein L23 (P48159) Evalue: 7e-35	50	1	10.7	0.84	11.15	-10.31
65	contig08967:1747:2187:3 No Annotation	270	6	52.1	7.35	18.25	-10.9
66	contig19869:105:422:3 similar to Muscle LIM protein 1 (P53777) Evalue: 8e-22	159	3	49.5	1.83	13	-11.17
67	contig22223:90:578:3  GG13930 [ <i>Drosophila erecta</i> ] (XP_001979164.1) Evalue:2e-14	337	11 (4)	36.4	11.43		-11.43

## Results and discussion

no.	Accession no./Protein description	Protein score	Peptide matches (unique peptides)	Protein coverage	emPAI (AS)	emPAI (EES)	emPAI (AS-EES)
68	contig06849:1:534:1 similar to Soma ferritin (P42577) Evalue: 2e-55	561	20 (9)	58.2	11.7		-11.7
69	contig24148:1:277:2 similar to 40S ribosomal protein S25 (Q8ISN9) Evalue: 7e-24	71	3 (2)	28.6	1.38	13.5	-12.12
70	contig17326:97:498:1 similar to Histone H2B (P17271) Evalue: 2e-44	96	3 (2)	18	0.53	12.67	-12.14
71	contig03062:1:281:2 No Annotation	166	16 (3)	39.8	25.06	37.22	-12.16
72	contig17480:2216:2605:3 similar to Profilin (P25843) Evalue: 2e-25	59	1	7.8	0.5	12.81	-12.31
73	contig16808:174:557:3 similar to 60S ribosomal protein L30 (P58374) Evalue: 7e-50	74	1	12.6	0.48	13.14	-12.66
74	contig24216:1:620:3 similar to Peptidyl-prolyl cis-trans isomerase (P25007) Evalue: 1e-75	373	12 (7)	41.5	3.18	16.32	-13.14
75	contig23887:1:845:2 No Annotation	799	53 (15)	51.2	13.39		-13.39
76	contig26856:74:535:2 similar to Nucleoside diphosphate kinase B (Q5RFH3) Evalue: 2e-64	203	4	31.4	2.54	16.55	-14.01
77	contig02295:455:757:2 hypothetical protein BRAFLDRAFT_69973 [Branchiostoma floridae] (XP_002591410.1) Evalue: 8e-04	290	32 (5)	52.5	58.29	72.73	-14.44
78	contig24583:1:349:2 similar to Histone H2A.x (P16104) Evalue: 8e-24	322	12 (5)	60.9	4.48	20.44	-15.96
79	contig22223:927:1238:3 No Annotation	275	11 (4)	39.8	16.59		-16.59
80	contig06938:1:111:1 similar to Superoxide dismutase [Cu-Zn] (O46412) Evalue: 2e-08	74	2	70.3	7.12	24.66	-17.54
81	contig18814:70:537:1 similar to 40S ribosomal protein S18 (Q8ISP0) Evalue: 4e-53	264	8 (6)	36.1	4.45	24.19	-19.74
82	contig04326:1:434:3 No Annotation	435	36 (9)	55.2	19.85		-19.85
83	contig28231:1:506:3 No Annotation*[IPR002252 Glycoside hydrolase, family 36]	343	10 (4)	36.9	20.21	40.43	-20.22
84	contig05681:124:251:1 similar to 60S ribosomal protein L26 (P61256) Evalue: 7e-12	102	4 (3)	64.3	6.21	26.47	-20.26
85	contig10844:1:307:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 7e-25	203	4	65.3	6.87	29.04	-22.17
86	contig26127:1:557:3 similar to Fatty acid-binding protein, adipocyte (O97788) Evalue: 7e-21	234	5 (4)	26.1	2.01	24.47	-22.46
87	contig18941:155:934:2 No Annotation*[IPR013248 Shr3 amino acid permease chaperone]	717	57 (11)	56	11.41	35.41	-24
88	contig08851:1:613:2 similar to 60S ribosomal protein L7 (O01802) Evalue: 4e-82	577	21 (14)	62.6	13.27	39.62	-26.35
89	contig13035:1:170:1 No Annotation	168	10 (2)	57.1	58.2	90.14	-31.94
90	contig20910:105:416:3 similar to Histone H4 (P62799) Evalue: 9e-40	87	2	17.5	3.27	37.24	-33.97
91	contig23734:1:153:2 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 6e-16	246	9 (4)	78	29.43	66.59	-37.16
92	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	338	26 (5)	64.2	37.64	76.29	-38.65
93	contig08235:1:820:2 No Annotation#[IPR008197 Whey acidic protein, 4-disulphide core]	719	23 (10)	45.6	15.66	57.82	-42.16
94	contig21510:1907:2014:1 similar to 40S ribosomal protein S30 (P62861) Evalue: 3e-05	226	7 (4)	77.1	43.57		-43.57
95	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	1689	126 (24)	67.5	60.6	107.82	-47.22
96	contig21262:473:652:1 No Annotation*[IPR000187 Corticotropin-releasing factor, CRF]	102	3	52.5	6.02	60.53	-54.51
97	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	914	47 (15)	62	47.92	106.16	-58.24
98	contig19498:1:162:1 No Annotation	202	4 (3)	58.5	6.02	69.57	-63.55
99	contig26443:1:303:1 No Annotation	437	84 (5)	44.6	54.39	126.67	-72.28
100	contig18537:1:1312:2 similar to apolipoprotein B [Strongylocentrotus purpuratus] (XP_800206.2) Evalue: 1e-07	1373	99 (21)	51.5	65.75	141.61	-75.86
101	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	810	72 (10)	51.4	134.37	219.67	-85.3
102	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	1321	128 (20)	76.3	107.89	206.56	-98.67
103	contig08625:1:110:2 No Annotation	264	17 (3)	69.4	175.51	275.13	-99.62



no.	Accession no./Protein description	Protein score	Peptide matches (unique peptides)	Protein coverage	emPAI (AS)	emPAI (EES)	emPAI (AS-EES)
104	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	793	52 (12)	75.3	108.34	227.19	-118.85
105	contig24531:1:590:3  PREDICTED: apolipoprotein B [Danio rerio] (XP_694827.3) Evalue:2e-05	888	30 (11)	77.6	38.74	162.83	-124.09
106	contig08235:860:1596:2 No Annotation	937	51 (14)	56.7	68.46	197.64	-129.18
107	contig10105:1:309:3 No Annotation	421	16 (5)	63.7	49.63	258.84	-209.21
108	contig04531:1:1454:1 similar to Apolipoproteins (Q9U943) Evalue: 7e-11	2337	168 (33)	68.6	148.6	434.48	-285.88
109	contig18438:8:349:2 No Annotation*[IPR001846 von Willebrand factor, type D]	484	42 (6)	61.1	164.93	535.33	-370.4
110	contig18794:1:101:3 No Annotation*[IPR002136 Ribosomal protein L4/L1e]	192	6 (5)	97	70.62	874.69	-804.07

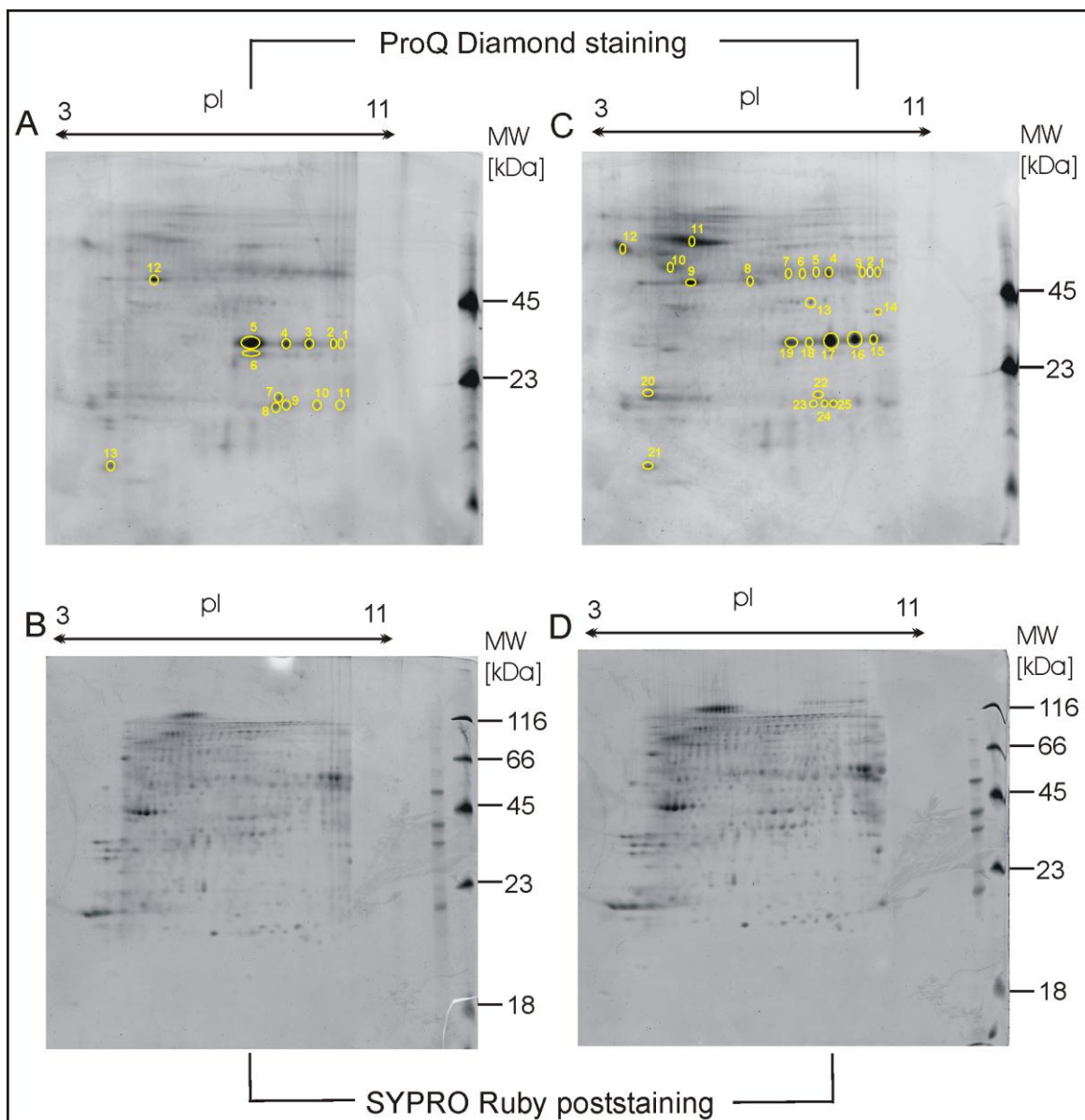
### 3.3 Detection of phosphoproteins on 2D gels

Protein phosphorylation is an important reversible event of biological processes in all cells that typically leads to changes of the conformation, activity and interactions of a protein within a very short time frame [57]. However, nothing is known about the role of posttranslational modifications such as phosphorylation in anhydrobiotic tardigrades. As has been shown in chapter 3.1.3 the comparative analysis of proteins identified only in tun state led to the assumption that phosphorylation could play an important role in anhydrobiosis. Therefore we performed first analyses for detecting and characterizing the phosphoproteins in active *versus* tun state. We developed optimized protocols for detecting phosphoproteins of tardigrades on a 2D gel using fluorescence staining (ProQ Diamond). ProQ Diamond is a commercially available dye used for phosphoprotein detection in particular on 2D gels. Detection of 1ng of multiply phosphorylated protein and 10 ng of a singly phosphorylated protein in gel is reported [92].

To compare the phosphoprotein profile of tardigrades in active *versus* tun state, we separated the protein extract from active and anhydrobiotic tardigrades on a 2D gel and performed ProQ Diamond staining (Figure 12A and C). As positive control we loaded PeppermintStick™ phosphoprotein molecular weight standard, which separated into two phosphorylated and four nonphosphorylated protein bands. To localize the detected phosphoproteins the gels were poststained with SYPRO Ruby (Figure 12B and D). Comparing ProQ Diamond images of phosphoprotein in active *versus* tun states, we could detect more protein spots in tun state, which corresponds to the higher level of phosphorylation. Interestingly, we detected a chain of protein spots (spots 1-6 in active state and spots 15-19 in tun state), which differ in their degree of phosphorylation in active *versus* tun state. It was surprising to see that these high phosphorylated proteins are weakly to detect (almost not detectable) by SYPRO Ruby staining. However, the same results were obtained analyzing another tardigrade species, *Ramazzottius varieornatus* (Dr. Kunieda Takekazu, personal communication).

A total of 25 spots in tun state and 13 spots in active state were selected and subsequently analyzed by ESI mass spectrometry on a LTQ-Orbitrap instrument. Since the level of protein phosphorylation at distinct amino acid residues is expected to be low, consequently several strategies are developed to enrich phosphopeptides. Immobilized metal ion affinity chromatography (IMAC) and titanium dioxide (TiO<sub>2</sub>) chromatography are two examples. To perform enrichment methods high amount of peptides is recommended, which demonstrates the major disadvantage of enrichment steps when the biological material is limited. In particular testing enrichment methods to select the optimal one for certain biological material needs high amount of protein extracts. In the present analysis we analyzed peptides without any enrichment steps, which resulted in identification of a few phosphopeptides. We could significantly detect

one phosphorylated peptide from each of following proteins: Gelsolin (contig03263:1:1127:3, spot T13 RQLSADAAFEK), Cathepsin L (contig17318:1:1144:2, T14, KLEPVLPEAVTSDEE) and one protein without annotation (contig08235:860:1596:2, A7, FGASSDEESDSFR). However, among identified proteins we found many proteins that have been reported to be phosphorylated such as vitellogenin, but we could not identify the corresponding phosphopeptides. The complementary approach using 1D gel electrophoresis in combination with high sensitive LC ESI-MS/MS is in progress and will probably deliver more information on phosphoproteins.



**Figure 12: Detection of phosphoproteins on 2D gels using ProQ Diamond staining.** Whole protein lysate of tardigrades in active (A) and tun (B) state were separated by pI in the first dimension using 13 cm long IPG strips with non-linear gradients from pH 3–11 and by MW in the second dimension. Gels were stained with ProQ Diamond for detecting phosphoproteins and poststained with SYPRO Ruby for detecting all proteins on the gels.

## 4 Conclusion

The present study demonstrates to the best of our knowledge the first comprehensive proteome analysis of tardigrades. Although tardigrades have been known for more than 300 years, there was almost no genomic and proteomic data available at the beginning of the project. Therefore we had to set up the basic protocols such as developing an optimal protein extraction method, which had to be compatible with all technologies used in this work like DIGE system. A complementary approach incorporating genomics and proteomics experiments has resulted in a broad coverage of proteins expressed in tardigrades. Since the availability of comprehensive, in ideal case species specific protein database is a prerequisite for protein identification, our genomic cooperation partner initiated the sequencing pipeline of tardigrades. The first protein database with 3318 protein sequences generated by Sanger sequencing allowed us developing the first proteome map of tardigrades [56]. The second protein database contained a high number of 24679 protein sequences and therefore represented a good starting point to undertake the comprehensive proteome profiling and quantification of proteins that are differentially expressed in different states. Using 1D gel electrophoresis in combination with high-throughput, high-sensitive nanoLC ESI-MS/MS on a LTQ-Orbitrap mass spectrometer we have identified more than 3000 unique proteins of *M. tardigradum* with high sequence coverage. A total of 1980 unique proteins has been identified in early embryonic state, 2245 unique proteins in active state, and 2118 unique proteins in anhydrobiotic state. Proteins known to be related to desiccation tolerance in other organisms were identified. These include proteins with antioxidant activity, chaperones in particular heat shock proteins, aquaporins and Late Embryogenesis Abundant (LEA) proteins.

The comparative proteome analysis of proteins expressed in adult tardigrades *versus* early embryonic state delivered knowledge about developmental proteins, which can be of importance in the field of developmental biology. In addition comparison of identified proteins only in active or tun states led to the assumption that processes such as phosphorylation and activation of intracellular signalling cascades are probably associated with anhydrobiosis. This is in accordance to the reports of observed changes in protein phosphorylation in plants which were exposed to water deficit, suggesting reversible phosphorylation as a regulator. In addition mitogen-activated protein kinases (MAPKs) and other kinases belonging to the MAPK cascade have been identified in plants in response to dehydration.

In the present study we performed two quantification approaches with regard to further in-depth quantitative analysis. Performing 2D DIGE technology we detected 57 spots, which were differentially regulated in active comparing to tun state. Although performing six technical replicates led to accurate and reliable statistical analysis, we faced the major limitation of DIGE technology, which is multiple protein identification in one spot. Therefore we had to include another quantification approach. For selecting the suitable quantification technology there were

some limitations, which had to be considered. Technologies such as SILAC and  $^{15}\text{N}/^{14}\text{N}$  metabolic labeling rely on metabolic incorporation of the isotopes and are suitable for cell culture and only in rare cases for whole organism because the whole food chain of the organism has to be considered for labeling. Furthermore the number of tardigrades cultivated in the laboratory is limited and only the homogenization of a high number of individuals results in enough protein amounts to perform experiments with biological replicates. This represents the major limitation in investigating tardigrades performing quantification approaches. Label-free technique using emPAI was used in the present study to achieve a simple and fast quantification. This semi-quantitative analysis was predominantly used for estimation of relative protein concentration to grouping the proteins into minor and major components. This method was approved by delivering consequential results in comparing early embryonic state *versus* adults. We could observe the up-regulation of specific protein families such as large lipid transfer (LLTP) superfamily and ribosomal proteins in early embryonic state. Vitellogenins, protein members of LLTP superfamily, are the major yolk proteins and serve as nutrition sources and therefore play important roles in embryogenesis. The high expression of ribosomal proteins in this state is due to the need of synthesis of diverse proteins needed for developing the mature organism. In addition we found proteins that are highly expressed in early embryonic state like small heat shock protein major egg antigen (p40) and soma ferritin, which probably represent potential markers for early embryonic state.

Since a majority of 1981 unique proteins overlap with both active and tun states, the detection of differences in abundance of single proteins are of major importance. Using emPAI we performed a semi-quantitative analysis of heat shock proteins in the active *versus* the anhydrobiotic state. The analysis delivered results that could be confirmed by the parallel gene expression analysis [55]. For analyzing the whole large scale data manually we selected a high cut-off of  $>10$  and  $<-10$  for  $\Delta\text{emPAI}$ . Under the selected conditions we observed proteins that show high differential regulation. Other probably important proteins, which show lower differential regulation, were not considered. The quantification of proteins can be performed automatically using diverse available softwares, which quantify proteins based on spectral counting. Scaffold Proteome Software is a robust program for label-free quantification that enables also the statistical analysis in case biological replicates are present. The MS/MS analysis of a replicate of each three states for analysis by Scaffold program is ongoing.

The proteome analysis of tardigrades from the first step of developing optimal extraction protocols to quantitative analysis of differentially expressed proteins established the basis for future investigations in the field of anhydrobiotic organisms en route to understanding survival mechanisms and eventually developing new methods for preservation of biological materials. In particular the role of up-regulated proteins in tun state, which could not be annotated by homology search, has to be investigated by analyzing their molecular function.

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## 6 Manuscripts

### 6.1

#### **Proteomic analysis of tardigrades: towards a better understanding of molecular mechanisms by anhydrobiotic organisms**

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# Proteomic Analysis of Tardigrades: Towards a Better Understanding of Molecular Mechanisms by Anhydrobiotic Organisms

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## Abstract

**Background:** Tardigrades are small, multicellular invertebrates which are able to survive times of unfavourable environmental conditions using their well-known capability to undergo cryptobiosis at any stage of their life cycle. *Milnesium tardigradum* has become a powerful model system for the analysis of cryptobiosis. While some genetic information is already available for *Milnesium tardigradum* the proteome is still to be discovered.

**Principal Findings:** Here we present to the best of our knowledge the first comprehensive study of *Milnesium tardigradum* on the protein level. To establish a proteome reference map we developed optimized protocols for protein extraction from tardigrades in the active state and for separation of proteins by high resolution two-dimensional gel electrophoresis. Since only limited sequence information of *M. tardigradum* on the genome and gene expression level is available to date in public databases we initiated in parallel a tardigrade EST sequencing project to allow for protein identification by electrospray ionization tandem mass spectrometry. 271 out of 606 analyzed protein spots could be identified by searching against the publicly available NCBI nr database as well as our newly established tardigrade protein database corresponding to 144 unique proteins. Another 150 spots could be identified in the tardigrade clustered EST database corresponding to 36 unique contigs and ESTs. Proteins with annotated function were further categorized in more detail by their molecular function, biological process and cellular component. For the proteins of unknown function more information could be obtained by performing a protein domain annotation analysis. Our results include proteins like protein member of different heat shock protein families and LEA group 3, which might play important roles in surviving extreme conditions.

**Conclusions:** The proteome reference map of *Milnesium tardigradum* provides the basis for further studies in order to identify and characterize the biochemical mechanisms of tolerance to extreme desiccation. The optimized proteomics workflow will enable application of sensitive quantification techniques to detect differences in protein expression, which are characteristic of the active and anhydrobiotic states of tardigrades.

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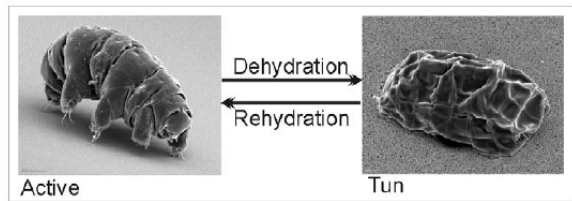
**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Many organisms are exposed to unfavourable, stressful environmental conditions, either permanently or for just certain periods of their lives. To survive these extreme conditions, they possess different mechanisms. One of amazing adaptation is anhydrobiosis (from the Greek for “life without water”), which has puzzled scientists for more than 300 years. For the first time the Dutch microscopist Anton van Leeuwenhoek (1702) gave a formal description of this phenomenon. He reported the revival of “animalcules” from rehydrated moss samples. In extreme states of dehydration, anhydrobiotic invertebrates undergo a metabolic dormancy, in which metabolism decreases to a non-measurable level and life comes to a reversible standstill until activity is resumed

under more favourable conditions [1]. One of the best known anhydrobiotic organisms are tardigrades. Tardigrades remain in their active form when they are surrounded by at least a film of water. By losing most of their free and bound water (>95%) anhydrobiosis occurs [2]. Tardigrades begin to contract their bodies and change their body structure into a so-called tun state (Figure 1). In the dry state these organisms are highly resistant to environmental challenge and they may remain dormant for a long period of time. Based on their amazing capability to undergo anhydrobiosis, tardigrades colonise a diversity of extreme habitats [3], and they are able to tolerate harsh environmental conditions in any developmental state [4]. Possessing the ability to enter anhydrobiosis at any stage of life cycle, tardigrades can extend their lifespan significantly [4,5]. Additionally, in the anhydrobiotic state, tardigrades are



**Figure 1. SEM images of *M. tardigradum* in the active and tun state.** Tardigrades are in the active form when they are surrounded by at least a film of water. By losing most of their free and bound water (>95%) anhydrobiosis occurs. Tardigrades begin to contract their bodies and change their body structure into a so-called tun. doi:10.1371/journal.pone.0009502.g001

extraordinary tolerant to physical extremes including high and subzero temperatures [6,7,8], high pressure [6,9], and extreme levels of ionizing radiation [10,11]. Interestingly, tardigrades are even able to survive space vacuum (imposing extreme desiccation) and some specimens have even recovered after combined exposure to space vacuum and solar radiation [12].

Anhydrobiosis seems to be the result of dynamic processes and appears to be mediated by protective systems that prevent lethal damage and repair systems. However, the molecular mechanisms of these processes are still poorly understood. Up to now investigations of mechanisms of desiccation tolerance have focused mainly on sugar metabolisms, stress proteins and a family of hydrophilic proteins called LEA (late embryogenesis abundant). The presence of non-reducing trehalose and its expression during anhydrobiosis has been reported for different anhydrobiotic species [13,14], which indicates the important role of trehalose in anhydrobiosis. However, the existence of anhydrobiotic animals that exhibit excellent desiccation tolerance without having disaccharides in their system [15,16] shows that sugars alone do not sufficiently explain these phenomena.

*Milnesium tardigradum* Doyère (1840) is a very well known species of carnivorous tardigrade. Different aspects of the life history of this species have been already described [17]. While some genetic studies of *M. tardigradum* exist [18] almost nothing is known about the proteome. Partial sequences of three heat shock protein (hsp70 family) genes and the housekeeping gene beta-actin have been described [18] and the relation of hsp70 expression to desiccation tolerance could be shown by real time PCR [18] and by de novo protein synthesis [6]. Since no trehalose could be detected in *M. tardigradum* [19], investigating proteins and posttranslational modifications is of particular importance to clarify surviving mechanisms during desiccation.

To gain insight into the unique adaptation capabilities of tardigrades on the protein level we aimed to establish a comprehensive proteome reference map of active *M. tardigradum* employing optimized protocols for protein extraction, generation of high-resolution 2D gels and high-throughput protein identification by electrospray ionization tandem mass spectrometry (ESI-MS/MS). The proteome reference map of *M. tardigradum* provides the basis for further studies in order to understand important physiological processes such as anhydrobiosis and stress resistance. The optimized proteomics workflow will enable application of sensitive quantification techniques to detect differences in protein expression, which are characteristic of active and anhydrobiotic states. Thus, our proteomic approach together with in-depth bioinformatic analysis will certainly provide valuable information to solve the over 300 years existing puzzle of anhydrobiosis.

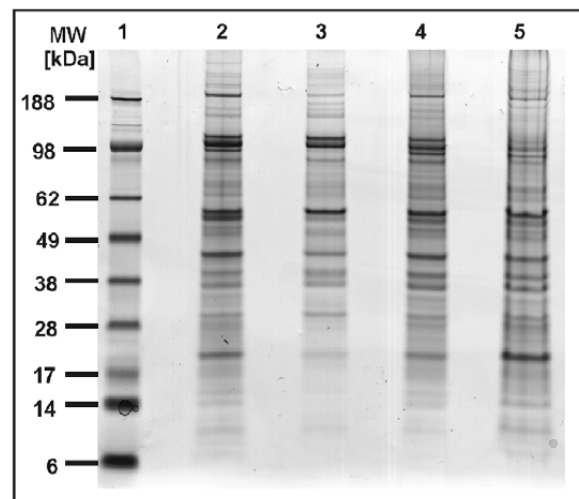
## Results

### Preparation of Protein Extracts from Active Tardigrades

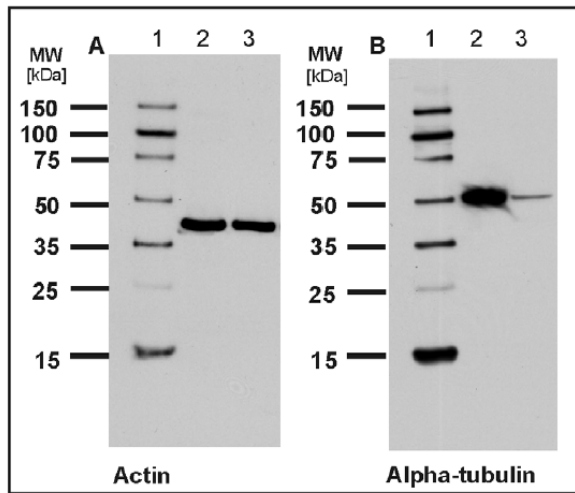
To establish and optimize a reliable and robust protocol for the extraction of proteins from tardigrades in the active state we applied different workup protocols and evaluated them by one-dimensional (1D) gel electrophoresis. Figure 2 shows the separation of protein extracts from whole tardigrades without any precipitation step (lane 2), after trichloroacetic acid/acetone precipitation (lane 3), after chloroform/methanol precipitation (lane 4) and after using a commercially available clean-up kit (lane 5). When using trichloroacetic acid/acetone precipitation we lost many proteins especially in the low molecular weight range. Chloroform/methanol precipitation and application of clean-up kit delivered satisfying results but also using the whole protein lysate directly without any further purification resulted in high yields across the entire molecular weight range. This workup protocol was therefore used throughout our proteome study. To evaluate the quality of our protocol especially with respect to proteolysis we performed Western blot analysis to detect any protein degradation. Since no proteins have been identified so far, we have chosen two polyclonal antibodies directed against the highly conserved proteins actin and alpha-tubulin. As shown in Figure 3A and 3B both proteins could be detected at their expected molecular weight at approx. 40 and 50 kDa, respectively, which is in agreement with the protein bands of the control lysate of HeLa cells. Importantly, no protein degradation could be observed during our sample preparation.

### Two Dimensional Gel Electrophoresis (2-DE)

The establishment of an optimized workup protocol was a prerequisite for high quality 2D gels from tardigrades in the active state. The proteomics workflow is depicted in Figure 4. One important step in the workflow is the collection and preparation of the samples. To avoid contamination with food-organisms,



**Figure 2. Comparison of different workup protocols for *M. tardigradum*.** Total protein extract of tardigrades in the active state was separated on a one-dimensional polyacrylamide gel. Lane 1: Rainbow molecular weight marker. Lane 2: Protein extract of whole tardigrades without any precipitation step. Lane 3: Protein extract after TCA precipitation. Lane 4: Protein extract after chloroform/methanol precipitation. Lane 5: Protein extract using clean-up kit. doi:10.1371/journal.pone.0009502.g002



**Figure 3. Analysis of protein degradation in total protein extracts of tardigrades by Western blot analysis.** Actin (A) and alpha tubulin (B) were used as marker proteins for the detection of proteolysis. Lane 1A and 1B: DualVue Western blotting marker. Lane 2A and 2B: Total protein extract of HeLa cells as control. Lane 3A and 3B: Total protein extract of *M. tardigradum*. Notably, no protein degradation was observed during the workup procedure. doi:10.1371/journal.pone.0009502.g003

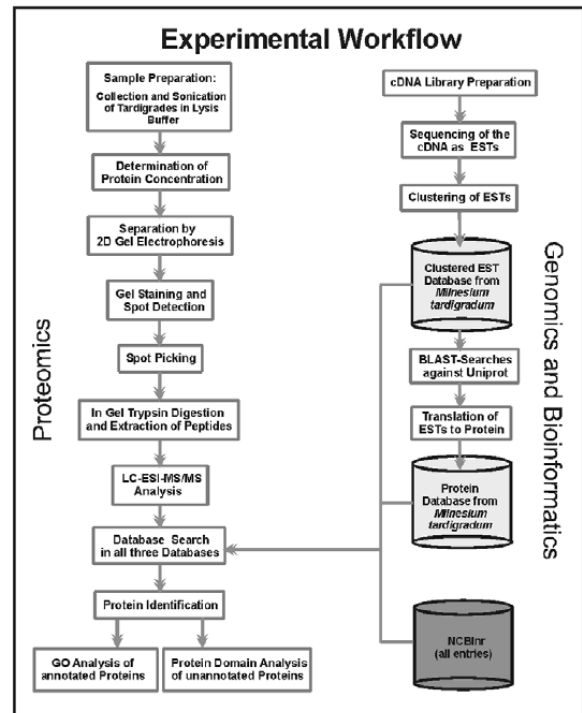
tardigrades were washed several times and starved over 3 days. Direct homogenization and sonication of deep-frozen tardigrades in ice cold lysis buffer without any previous precipitation step yielded protein extracts which were separated by high resolution 2D gel electrophoresis. For maximal resolution of protein spots and high loading capacity (330 µg proteins) we used pI 3–11 NL strips (24 cm) for the first dimension. Thus, high resolution separation could be achieved in the acidic as well as in the basic pH range as shown in the image of the silver stained preparative gel of whole protein extract (Figure 5).

Approximately 1000 protein spots were automatically detected on the 2D gel image using the Proteomweaver image software. A total of 606 protein spots were picked from the silver stained gel. These spots were digested with trypsin and after extraction of the tryptic peptides from the gel plugs peptide mixtures were analyzed by nanoLC-ESI-MS/MS.

**Protein Identification**

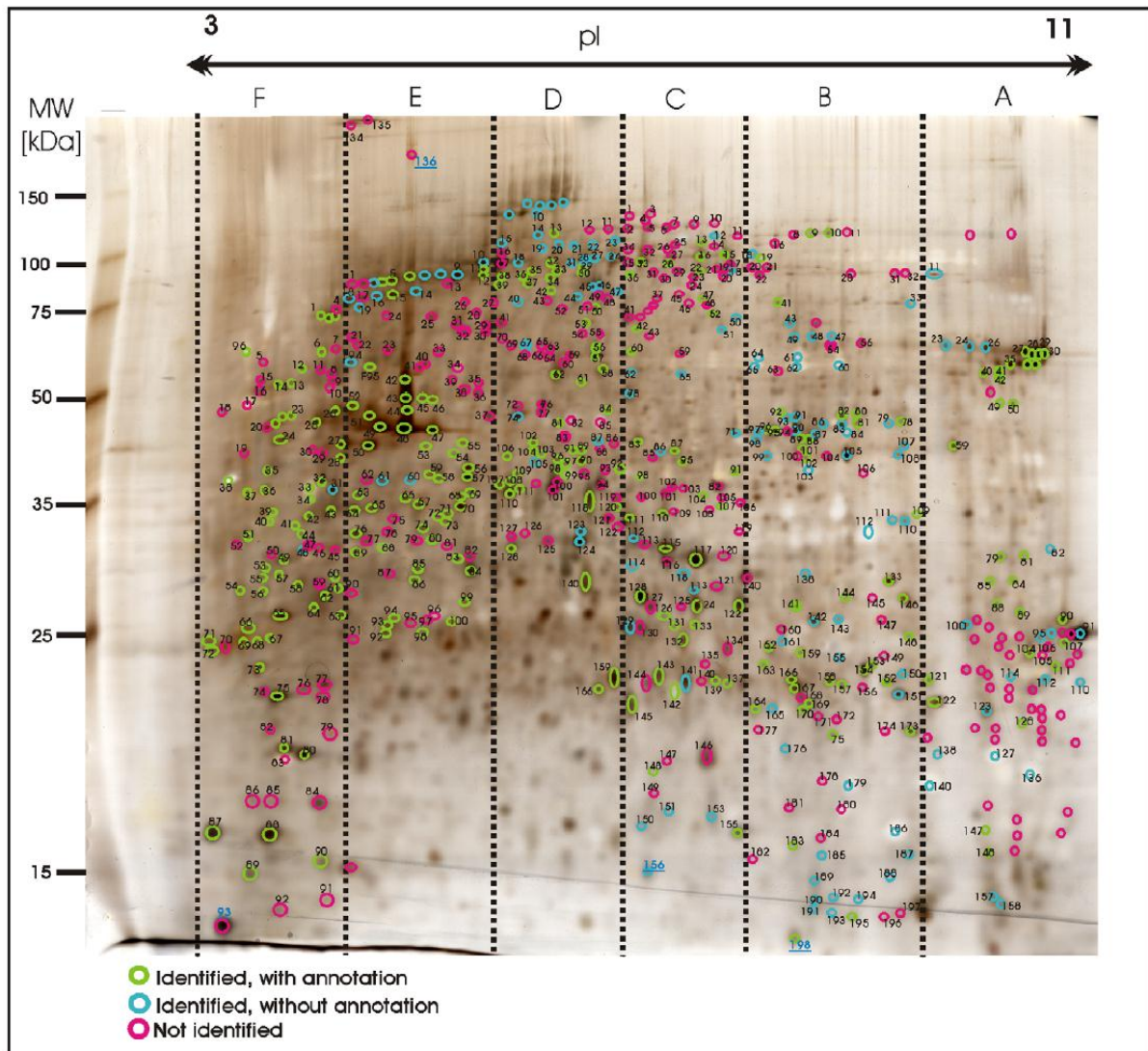
Identification of proteins depends on the representation of the sequence or a close homologue in the database. Since almost no genome or EST sequences of *M. tardigradum* are available to date in public databases we initiated the tardigrade EST sequencing project as outlined in figure 4 (Mali et al, submitted data). A cDNA library was prepared from tardigrades in different states (active, inactive, transition states). The cDNAs were sequenced as ESTs and clustered. Thereby, we obtained a nucleotide database containing 818 contigs and 2500 singlets. cDNA sequencing and generation of ESTs are still ongoing, thus the sequence coverage of *M. tardigradum* in the database is incomplete.

For protein identification we used the following databases: the database of *M. tardigradum* containing the clustered ESTs as outlined above, the tardigrade protein database, which was translated from the clustered EST database and thus represents a subdatabase containing only annotated proteins with known function and the publicly available NCBIInr database. The selected



**Figure 4. The experimental workflow to developing the proteome map.** Tardigrades were sonicated directly in lysis buffer. Total protein extracts were separated by two-dimensional gel electrophoresis. After silver staining protein spots were picked and in-gel digested with trypsin. MS/MS data obtained by LC-ESI-MS/MS analysis were searched against the NCBIInr database, the clustered tardigrade EST database and the tardigrade protein database. Identified proteins with annotation were classified in different functional groups using the Blast2GO program. Identified proteins without annotation were analysed with the DomainSweep program to annotate protein domains. doi:10.1371/journal.pone.0009502.g004

606 spots from the 2D gel correspond to some highly expressed proteins, but mostly to spots in the medium and low expression range. A total of 271 spots could be identified from the tardigrade protein and the NCBIInr databases. Figure 6 demonstrates how identified proteins are distributed among these two databases. 56 unique proteins were successfully identified by searching the NCBIInr database. It concerns proteins which are either highly conserved among different species e. g. actin or protein entries from *M. tardigradum* which are already available in the NCBIInr database e.g. elongation factor 1-alpha. Further 73 unique proteins could be identified by searching the tardigrade protein database and another 15 unique proteins were present in both databases. Identical proteins that were identified from several spots were included only once in the statistics to avoid bias. Thus, the combination of the two databases was sufficient for the identification of 144 unique proteins. The corresponding protein spots are indicated by green circles in the 2D reference map shown in Figure 5. Table 1 shows an overview of identified proteins with annotation in different functional groups. In addition, detailed information about each of the identified 144 proteins including spot number, protein annotation, accession number (NCBIInr and Tardigrade specific accession number), total protein score, number of matched peptides, peptide sequence and sequence coverage is



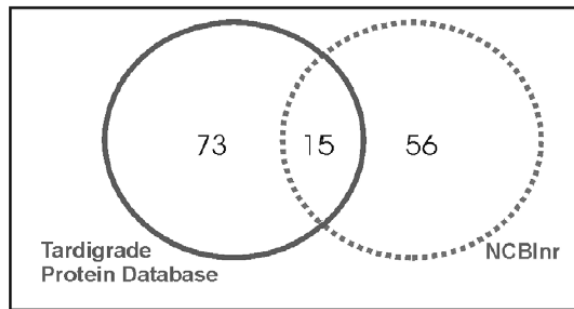
**Figure 5. Image of a preparative 2D-gel with selected analysed protein spots.** Total protein extract of 400 tardigrades in the active state corresponding to 330  $\mu$ g was separated by high resolution two-dimensional gel electrophoresis. Proteins were visualised by silver staining. Three different categories are shown: Identified proteins with functional annotation are indicated in green, identified proteins without annotation are indicated in blue and not yet identified proteins are indicated in red. doi:10.1371/journal.pone.0009502.g005

listed in Table 2. The individual ion score is included in brackets at the end of every peptide sequence. Following ion scores indicate a significant hit ( $p < 0.05$ ):  $>53$  for NCBI searches,  $>14$  for searches in the tardigrade protein database and  $>27$  for searches in the EST clustered database. Identical proteins identified in different spots are listed only once in Table 2. In these cases the spot with the highest protein score (in bold) is ranked at the top whereas the other spots are listed below. All further information such as accession numbers, peptide sequences and sequence coverage refer to the top-ranked spot.

The 15 proteins which were identified in both databases are indicated with asterisk (e.g. spot A30\*) and both accession numbers are listed. In these cases the listed peptide sequences belong to the hit with the highest score. Protein spots below the bold one are marked with  $\circ$ , when only found in the NCBI

database or marked with  $\wedge$ , when only found in the tardigrade protein database.

Furthermore we were able to identify additional 150 protein spots by searching MS/MS data in the clustered EST database of *M. tardigradum*. These 150 proteins correspond to 36 unique contigs and ESTs. The protein information is listed in Table 3 and the protein spots are indicated by blue circles in the 2D reference map (Figure 5). Unfortunately, it was not possible to annotate them when performing a BLAST search. For these proteins of unknown function more information could be obtained by applying protein domain annotation methods. We ran all proteins through the DomainSweep pipeline which identifies the domain architecture within a protein sequence and therefore aids in finding correct functional assignments for uncharacterized protein sequences. It employs different database search methods to scan a number of



**Figure 6. Comparison of database performance for protein identification.** Protein spots were analysed by nanoLC-ESI-MS/MS and searched against the NCBI nr database and the tardigrade protein database. The diagram illustrates the number of positive identifications in the respective database and the overlap between the two databases. doi:10.1371/journal.pone.0009502.g006

protein/domain family databases. 2 out of the 36 unique proteins gave a significant hit, whereas 28 proteins were listed as putative and 6 proteins gave no hit at all.

In addition, we analyzed further 185 protein spots, which are indicated with red colour in Figure 5. Despite high quality MS/MS spectra, it was not possible to identify these protein spots in either of the databases used in our study.

In summary, we identified 421 (69.5%) out of 606 protein spots which were picked from the preparative 2D gel. 271 spots yielded 144 unique proteins with distinct functions whereas 150 spots were identified as proteins with yet unknown functions.

### Functional Assignment of Proteins

The 144 unique proteins with annotation were further analysed using the Blast2GO program, which provides analysis of sequences and annotation of each protein with GO number to categorize the proteins in molecular function, biological process and cellular component. By analysing the proteins on the GO level 2 in the category molecular function we received a total of 9 subgroups as shown in Figure 7, upper middle chart. The majority of the identified proteins exhibit either binding (45%) or catalytic activity (33%). A more detailed analysis (GO level 3) revealed that 39% of the proteins with catalytic activity are involved in hydrolase activity (Figure 7, upper right chart) and 38% of binding proteins bind to other proteins (Figure 7, upper left chart).

Identified proteins are involved in diverse biological processes. A total of 16 subgroups of biological processes are represented (Figure 7, lower middle chart). 23% are involved in cellular processes and 18% in metabolic processes. Within the cellular processes a majority of 20% of tardigrade proteins are involved in cellular component organization and biogenesis. Within the metabolic processes 28% of proteins are involved in cellular metabolic processes, 26% in primary metabolic processes and 21% in macromolecule metabolic processes (Figure 7, lower right chart). A detailed GO description of all identified and annotated tardigrade proteins is included in Table S1.

### Identified Proteins and Protein Families

In our proteomic study several heat shock proteins have been identified, namely hsp-1 (spot F27), hsp-3 (spot F21), hsp60 (spot F57), hsp70 (spot B146, B173, C131, C133), hsp82 (spot F13), hsp86 (spot F24, F25), hsp90 alpha (spot E64), hsp90 beta (spot F24) and hsp108 (spot F12). Hsp70 is already described in *M. tardigradum* as a molecular chaperone which could play a role in desiccation

tolerance [18]. Hsp60 could be identified in spot F57 when searching the corresponding MS/MS data against the NCBI nr database. No hit was obtained in the tardigrade EST or protein database which is surprising, because hsp60 is an abundant protein.

Several protein spots have been identified as cytoskeletal proteins, including actin as most abundant protein spot (E48) on the 2D gel and tubulin. Actin and tubulin are highly conserved proteins and were used to control proteolytic degradation during our workup procedure by Western blotting. Four different actin proteins are found by MS/MS analysis, which play important roles in muscle contraction, cell motility, cytoskeletal structure and cell division. Tubulin is a key component of the cytoskeletal microtubules. Both alpha- and beta-tubulin could be identified on the 2D gel in spot D107, D110 and F6. Further proteins involved in motor activity and muscle contraction were found, namely tropomyosin (e.g. spot F35), myosin (e.g. spot F81), annexin A6 (e.g. spot D90) and myophilin (e.g. spot A128), which is a smooth-muscle protein and was described in the tapeworm *Echinococcus granulosus* [20].

In addition, several proteins have been identified which are known to have important roles in embryonic or larval development. Mitochondrial malate dehydrogenase precursor (e.g. spot B109), vitellogenin 1 and 2 (e.g. spot D62 and B88), GDP-mannose dehydratase (spot C87), protein disulfide isomerase 2 (e.g. spot F3), hsp-3 (spot F21), hsp-1 (spot F27), tropomyosin (spot F35) and troponin C (spot F87) belong to this group of proteins. Vitellogenin, a major lipoprotein in many oviparous animals, is known as the precursor of major yolk protein vitellin [21]. Vitellogenin is a phospholipo-glycoprotein which functions as a nutritional source for the development of embryos [22]. During developing oocytes vitellogenin and vitellin are modified through cleavage and by different posttranslational modifications (PTMs) like glycosylation, lipidation and phosphorylation. Interestingly we could identify vitellogenin in several spots on the 2D gel showing vertical (pI) shifts most probably caused by PTMs.

Peroxiredoxins identified first in yeast [23] are conserved, abundant, thioredoxin peroxidase enzymes containing one or two conserved cysteine residues that protect lipids, enzymes, and DNA against reactive oxygen species. Different isoforms of peroxiredoxins could be identified on the 2D gel: peroxiredoxin-4 (spot C132), peroxiredoxin-5 (spot B183) and peroxiredoxin-6 (spot D159). An important aspect of desiccation tolerance is protection against free radicals [24,25]. Notably, the expression of 1-cysteine (1-Cys) peroxiredoxin family of antioxidants is reported in *Arabidopsis thaliana* and is shown to be related to dormancy [26]. Our results show the presence of important antioxidant systems, including superoxide dismutase (SOD) and peroxidases. Additionally different forms of glutathione S-transferases (spot A122, B153, B166, B169, D166, and D159) could be identified. Glutathione transferases (GSTs) constitute a superfamily of detoxifying enzymes involved in phase II metabolism. Detoxification occurs by either glutathione conjugation, peroxidase activity or passive binding [27]. Furthermore GSTs have cellular physiology roles such as regulators of cellular pathways of stress response and housekeeping roles in the binding and transport of specific ligands [28]. The consequence of this diversity in role is the expression of multiple forms of GST in an organism. It has been shown that the expression of the different isoenzymes is highly tissue-specific [29], and this heterogeneity of GSTs may be further complicated by posttranslational modifications such as glycosylation [30].

Some protein spots were identified as calreticulin (e.g. spot F14) which is a  $\text{Ca}^{2+}$ -binding protein and molecular chaperone. Calreticulin is also involved in the folding of synthesized proteins and glycoproteins [31].

**Table 1.** Overview of identified proteins classified in different functional groups.

<b>Cytoskeleton elements and modulators</b>	<b>Enzymes</b>	<b>Proteases and protease inhibitors</b>
Alpha-III tubulin	Glucan endo-1,3-beta-glucosidase	Cathepsin K
Beta-tubulin class-IV	Prostatic acid phosphatase	Cathepsin Z
Beta-tubulin class-I	Adenylate kinase isoenzyme 1	Cathepsin L1
Actin	Peptidyl-prolyl cis-trans isomerase	Nepriylsin-2
Actin-5C	Glutamate dehydrogenase	Peptidase M17 precursor
Beta actin	Lysosomal acid phosphatase	Actinidain
Alpha actin	Mitochondrial malate dehydrogenase	Plasminogen
Actin, muscle-type (A2)	Arginine kinase	Aspartic protease inhibitor 8
muscle actin	Aconitase, mitochondrial	AFG3-like protein 2
Similar to alpha actinin CG4376-PB	Transaldolase	26S proteasome non-ATPase regulatory subunit 8
Myophillin	Aldolase A protein	Rab GDP dissociation inhibitor beta
Tropomyosin-1, isoforms 9A/A/B	Protein disulfide isomerase-3	Gamma-glutamyltranspeptidase
Tropomyosin	Matrix metalloproteinase-17	<b>Response to stress or heat</b>
Myosin regulatory light polypeptide 9	Mitochondrial long-chain enoyl-CoA hydratase/3-hydroxycyl-CoA	NADP-dependent isocitrate dehydrogenase
Myosin, essential light chain	Dehydrogenase alpha-subunit	Heat shock 70 kDa protein II
<b>Heat shock proteins</b>	Peroxidase	similar to heat shock cognate 70 protein isoform 2
Heat Shock Protein family member (hsp-3)	Methylmalonate-semialdehyde dehydrogenase	Short-chain dehydrogenase/reductase SDR YhdF
Heat Shock Protein family member (hsp-1)	Thioredoxin reductase 1	Aspartic protease inhibitor 8
Hsp 60	Succinyl-CoA ligase [GDP-forming] subunit beta, Mitochondrial E	UspA
Hsp 70	GTP-specific succinyl-CoA synthetase beta subunit	Rubber elongation factor protein (REF) (Allergen Hev b 1)
Heat shock cognate 70	Glycosyl transferase	Small rubber particle protein (SRPP) (22 kDa rubber particle protein)
Heat shock cognate 70 protein isoform 2	DEAD-box family (SNF2-like) helicase	Heat shock protein 90-beta
Heat shock 70 kDa protein II (HSP70 II)	Cysteine conjugate beta-lyase	Heat shock protein 83
Hsp 90-beta	26S proteasome non-ATPase regulatory subunit 13	Heat shock protein 60
Hsp90-alpha	GH19645	<b>Other Proteins</b>
Hsp90	<b>Glycolysis</b>	Translationally-controlled tumor protein homolog
Hsp 82	Glyceraldehyde-3-phosphate dehydrogenase	Elongation factor 1-alpha
Hsp 83	Triosephosphate isomerase	Elongation factor 1 gamma
Hsp108	Enolase	Elongation factor 2
Protein lethal(2)essential for life (member of Hsp20 family)	Phosphoglycerate kinase	Angiotensin-related protein 1
<b>Embryonic/larval development</b>	<b>Transporters</b>	Spaghetti CG13570-PA
Vitellogenin-1	H(+)-transporting ATP synthase	Prohibitin
Vitellogenin-2	ATP synthase subunit d, mitochondrial	Proteasome subunit alpha type-4
Protein disulfide-isomerase 2	ATP synthase beta subunit	40S ribosomal protein S12
Heat Shock Protein family member (hsp-3)	Mitochondrial ATP synthase alpha subunit precursor	Periostin
Heat Shock Protein family member (hsp-1)	Annexin A6	Acetylcholine receptor subunit alpha-L1
Troponin C	<b>Antioxidant proteins</b>	Nucleosome remodelling factor – 38kD CG4634-PA
Putative LEA III protein isoform 2	Thiol-specific antioxidant protein	Coiled-coil domain-containing protein 25
GDP-Mannose Dehydratase	Superoxide dismutase [Cu-Zn]	Calreticulin
Tropomyosin	Peroxiredoxin-5, mitochondria	Lipoprotein-related protein
<b>Dormancy related protein</b>	Peroxiredoxin-4	14-3-3 protein beta/alpha-2 (Protein 14-3-3B2)
Putative LEA III protein isoform 2	Glutathione S-transferase	60S ribosomal protein L26-1
	Peroxiredoxin-6	Histone H4
		Histone H2B.2

Identified proteins with annotation are listed in 8 different groups with majority in protein enzymes. We also identified many heat shock proteins and proteins, which are involved in embryonic development, response to stress/heat and dormancy.  
doi:10.1371/journal.pone.0009502.t001



**Table 2.** Identified proteins with annotation.

Spot no.	Protein name	NCBIr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
A30*	elongation factor 1-alpha [Milesium tardigradum]	gi 4530101	EZ048811	544	5	K.YAWVIDK.I (23) R.IPLQDVK.I (52)	51%
A27*, A28*, A29*, A32*, A33*, A35*, A40*, A41*, A42*, A49*, A50*, A59*, A81*, A84^, A85^, A88*, A107^, B78^, B80*, B81*, C28*, C36*, D120*						K.IGGIGTVFVGR.V (56) R.EHALLAYTLGVK.Q (65) K.YYVTIIDAPGHR.D (67) K.MDSSEPFSEDR.F + Oxidation (M) (72) R.NGYTPVLDCHTAHACK.F (18) K.MDSSEPFSEDRFNEIVK.E (12) K.TLLEALDSISPPARPDKPLR.I (69) R.VETGVIKGMVVFAPFTGLTEVK.S (34) K.NMITGTSQADCAVLVTPAPGFEAGISK.N (16) K.SGDAALVNIPTKLCVFAFSEYPPVIGR.- (45) R.VFTSDVDFNR.C (35)	
A79	DB:Swissprot Frame:3 orf:3 Homolog:Angiopoietin-related protein 1 Evaluate:1e-29 Bitscore:130	EZ048825		35	1	K.LSEEFVR.D (13) R.WSDVTRPGCK.G (46) R.NSWGPNWANK.G (33)	5%
A84	DB:Swissprot Frame:1 orf:8 Homolog:Cathepsin K Evaluate:1e-16 Bitscore:73.6	GH986829		58	1		16%
A85	DB:Swissprot Frame:1 orf:7 Homolog:Actinidain Evaluate:1e-11 Bitscore:70.5	EZ048769		33	1		18%
A90	DB:Swissprot Frame:3 orf:3 Homolog:Glucan endo-1,3-beta-glucosidase A1 Evaluate:1e-23 Bitscore:110 DB:Trembl Frame:3 orf:1 Homolog:GF11309 Evaluate:1e-06 Bitscore:57.8	EZ048807 EZ048774		28 192	1 3	K.EMFSVNDSPNKR.I + Oxidation (M) (28) R.GAVSCIDSFVNR.C (68) R.FNFQPPASILQDR.K (74) K.DLSLQTFTELCTR.S (49) K.ILGAGFSDTFADLLR.T (55)	5% 20%
	spaghetti CG13570-PA [Drosophila melanogaster]	gi 17864228		55	1		2%

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/ significant peptides	MS/MS peptide sequence (Indiv. Ion score)	Sequence coverage
A104	DBSwissprot Frame:2 of:3 Homolog:Prostatic acid phosphatase Evalue:3e-24 Bitscore:112		GH986832	162	4	R. YSSYLGPK. F (53) K. TVVNNELGQITSK. G (56) K. FSIPEVLIVSSAVER. A (37) R. AVQSTLVNAAAGLFFPSGDTIWNSSSEIGK. T (17)	48%
A89				200	4	R. YSSYLGPK. F (44) R. SPIFFTFDYGK. T (63) K. FSIPEVLIVSSAVER. A (48) K. GMQMYQLGQLSAR. Y + 2 Oxidation (M) (45) K. FNASQLITQR. Q (54) R. VLPSICNEVIK. G (67)	37%
A111*	prohibitin [Aedes aegypti]	gi 157131967	(EZ048795)	121	2	R. VLDALGTPIDGK. G (54) K. CAVVDVPPFGAK. G (53) K. GFLGFGVDVFPADMGTER. E + Oxidation (M) (4)	7%
A121	mitochondrial ATP synthase alpha subunit precursor [Strongylocentrotus purpuratus]	gi 47551121		97	1	R. VLDALGTPIDGK. G (54) K. CAVVDVPPFGAK. G (53) K. GFLGFGVDVFPADMGTER. E + Oxidation (M) (4)	3%
ZK829.4	[Caenorhabditis elegans]	gi 17544676		57	1	K. YLLEIVGK. Y (43) K. SYDQFETQPK. W (31) K. QYQNLADYHK. R (5) R. IMYMSQDFEK. E + Oxidation (M) (32) K. HYDMFSQFLGNK. K + Oxidation (M) (32) K. LTQSTALIMHFLAR. K + Oxidation (M) (70) K. QSLGLFFPNIPIYYIDGNFK. I (2)	5%
A122, B170, B175	DBSwissprot Frame:1 of:3 Homolog:Glutathione S- transferase 1 Evalue:1e-39 Bitscore:164		EZ048812	439	8	K. YLLEIVGK. Y (43) K. SYDQFETQPK. W (31) K. QYQNLADYHK. R (5) R. IMYMSQDFEK. E + Oxidation (M) (32) K. HYDMFSQFLGNK. K + Oxidation (M) (32) K. LTQSTALIMHFLAR. K + Oxidation (M) (70) K. QSLGLFFPNIPIYYIDGNFK. I (2)	45%
B153	DBSwissprot Frame:3 of:2 Homolog: Glutathione S- transferase Evalue:5e-44 Bitscore:177		EZ048805	260	7	K. YLLEIVGK. Y (43) K. SYDQFETQPK. W (31) K. QYQNLADYHK. R (5) R. IMYMSQDFEK. E + Oxidation (M) (32) K. HYDMFSQFLGNK. K + Oxidation (M) (32) K. LTQSTALIMHFLAR. K + Oxidation (M) (70) K. QSLGLFFPNIPIYYIDGNFK. I (2)	55%

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/ significant peptides	M/S/MS peptide sequence (Indv. Ion score)	Sequence coverage
<b>B166</b>	DB:Swissprot Frame:2 orf:1 Homolog: Glutathione S- transferase Evalue:3e-43 Bitscore:176		EZ048770	213	6	K.TEERQQCDMVEGALSDFR.Q + Oxidation (M) (25) K.QYLLGSDIK.Y (25) R.YLLEYGEEK.Y (43) K.LMYGSQDFEK.D + Oxidation (M) (23) K.LTQSNALHHLAR.K (63) K.LMYGSQDFEKD.S + Oxidation (M) (41) K.SEEEEQQCDMIEGALHDFR.M + Oxidation (M) (18)	31%
<b>B169</b>	DB:Swissprot Frame:1 orf:3 Homolog: Probable glutathione S-transferase 9 Evalue:8e-24 Bitscore:110		GH986911	96	2	R.LLFAAADQK.Y (48) K.VLAQTTSIVR.Y (48)	20%
<b>D166</b>	DB:Swissprot Frame:3 orf:3 Homolog: Glutathione S- transferase 1 Evalue:3e-30 Bitscore:131		GH986673	48	2	K.DMLVAMQR.W (14) K.LKGEELMDYMK.D (11) K.DQTFYGLPFILEVDGDK.I (23)	18%
<b>D159</b>	DB:Swissprot Frame:1 orf:3 Homolog: Probable glutathione S-transferase 6 Evalue:2e-34 Bitscore:146		EZ048796	405	8	R.IIFDENDK.S (56) R.SFQFFEK.Y (31) R.IIFDENDKSK.G (43) K.FTEATFPASIR.S (47) R.KFTEATFPASIR.S (63) R.LIFHGTFEDFEDVR.I (61) R.TEEALADSVVDTATNDILGDLIR.I (48) K.SRTEALADSVVDTATNDILGDLIR.I (58)	33%
<b>A128</b>	DB:Swissprot Frame:3 orf:2 Homolog:Myophilin Evalue:1e-33 Bitscore:143		EZ048783	273	6	R.NFSDEQLR.Q (35) R.LANEIQGSIR.K (43) R.AAEVCEWVVK.I (38) K.ILGENVLSTSGK.M (84) R.QGETMISLQYGSNK.G (48) K.QNLNAVVICLESIGLR.K (25) K.GFLIDGFPR.E (19)	40%
<b>A147</b>	DB:Swissprot Frame:3 orf:2 Homolog:Adenylate kinase isoenzyme 1 Evalue:6e-42 Bitscore:171		EZ048787	19	1		4%
<b>A148</b>	DB:Swissprot Frame:1 orf:1 Homolog:Peptidyl- prolyl		EZ048822	140	3	K.TSKFVVIADCGQL.- (34)	25%

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/ significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
	cis-trans isomerase	Evalue:2e-75 Bitscore:282				K.TSRPVIADCGQL.- (59) K.HVVFQVTEGLDIVK.K (49)	
B19	DB.Swissprot Frame:2 orf:1 Homolog: Elongation factor 2 Evalue:1e-72 Bitscore:271		GH986944	22	1	R.VFSGTVQTGQK.V (22)	6%
B41	PREDICTED: similar to CG8036-PB, isoform B	gi 66503776		125	1	K.LDSDLLEGHPTFR.L (48)	3%
B82	isoform 2 [Apis mellifera] glutamate dehydrogenase, short peptide [Drosophila melanogaster]	gi 458803		116	1	R.KLSDLEGHPTFR.L (53) K.IIAEAANGFTFAADK.I (50)	9%
B88	DB.Swissprot Frame:2 orf:1 Homolog: Vitellogenin-2		EZ048823	200	4	K.TFIVQGFQNVGLHPTFR.Y (62) K.VSMINLR.L (41)	14%
B9, B10, B89, B96, C22, C36, C47, C83, C87, C117, C122, C124	Evalue:1e-14 Bitscore:81.6					R.AEDEYEWRS.A (40) K.TIVVLPFSIYYK.N (51)	
B92	NADP-dependent isocitrate dehydrogenase [Homo sapiens]	gi 3641398		69	0	K.IMVLFQHSIEITAPQGR.T + Oxidation (M) (68) K.DIFQEIYDK.Q (42)	4%
B95	DB.Swissprot Frame:1 orf:1-3 Homolog;Uncharacterized protein C3orf33 homolog		GH986689	17	1	R.FKDIQEIYDK.Q (16) R.KDPEAMNETAK.W + Oxidation (M) (17)	6%
B102	Evalue:3e-06 Bitscore: DB.Swissprot Frame:3 orf:1 Homolog;Lysosomal		EZ048780	90	2	K.FLEPVTVPR.A (52)	46%
B101	acid phosphatase Evalue:3e-10 Bitscore:65.1					K.FILYSAHNTISALLAAFK.A (28)	
109	mitochondrial malate dehydrogenase precursor	gi 33439518		218	3	K.NNNVFDAPFTVIFPCSEFCFLDQLR.K (10) R.IQDAGTEVVNAK.A (64)	11%
A121	[Branchiostoma belcheri tsingtaunense]					R.DDLFNTNASIVR.D (66) K.AGAGSATLSMAYAGAR.F (87)	
B146	hypothetical protein TRIADDRAFT _63625	gi 195999922		342	4	R.VEIIANDQGNR.I (38)	8%
B141, B144	[Trichoplax adhaerens]					R.ITFYSVVAFTADGER.L (91) R.IINEPTAAAIAYGLDK.K (79)	

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. ( )	Tardigrade specific Accession no. (contig/EST) ( ^ )	Total protein score	No. of unique/ significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
						K.NQLTSNPENVFVDVK.R (72)	
						R.IINEPTAAAIAYGLDKK.E (62)	
<b>B173</b>	heat shock cognate 70 [Aedes aegypti]	gi 94468966		235	2	K.IQVEYKGETK.N (38) K.MKETAAYLQK.T + Oxidation (M) (62)	8%
						R.IINEPTAAAIAYGLDKK.K (28)	
						K.STAGDTHLGGEDFDNR.I (50)	
<b>C131</b>	Heat shock 70 kDa protein II (HSP70 II)	gi 123622		154	2	R.IINEPTAAAIAYGLDKK.T (57) K.ETAAYLQK.E (34)	8%
						K.VEIANDQGNR.T (60)	
						R.TTPSYVAFDTER.I (60)	
<b>C133</b>	PREDICTED: similar to heat shock cognate 70 protein isoform 2 [Acyrtosiphon pisum]	gi 193603576		153	2	K.VEIANDQGNR.T (60) R.TTPSYVGFDTTER.I (62)	8%
						R.IINEPTAAAIAYGLDKK.K (16)	
						K.STAGDTHLGGEDFDNR.M (16)	
<b>B148</b>	DB:Swissprot Frame:3 orf:2 Homolog:Malate dehydrogenase, cytoplasmic Value:3e-66	GH986821		179	3	R.AIGQMAIQLK.N (52) K.DQGSALNQYAK.K (60)	24%
						K.IILVGNPANTNAYILSHYAPSLK.E (67)	
<b>B152</b>	H(+)-transporting ATP synthase [Rattus norvegicus]	gi 57029		92	1	K.LELAQYR.E (31)	8%
<b>B164*</b>	ATPase subunit [Beta vulgaris subsp. Vulgaris]	gi 11263	(EZ048779)	64	0	R.EAYPGDVYLSHR.I (61) K.LELAQYR.E (34)	4%
<b>B167*</b>	DB:Swissprot Frame:1 orf:1 Homolog:Arginine kinase Value:5e-90 Bitscore:295	(gi 124264768)	EZ048827	254	6	R.GIRPAINVGLSVSR.V (29) R.FLQAAQAVR.F (41)	33%
B133 <sup>^</sup> , B157 <sup>^</sup> , B159 <sup>*</sup> ,						K.LIDDHFLFK.E (39)	
B162 <sup>^</sup> , C91 <sup>*</sup> , C98 <sup>^</sup> , C104 <sup>^</sup> ,						K.LNFPNPEGK.Y (60)	
C107 <sup>^</sup> , C137 <sup>^</sup> , C142 <sup>^</sup> ,						R.KYMFELIQK.I + Oxidation (M) (29)	
D98 <sup>^</sup> , D159 <sup>^</sup>						R.SLQGFPPNPLINEQQYK.E (30)	

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (†)	Tardigrade specific Accession no. (contig/EST) (Δ)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Incl. Ion score)	Sequence coverage
B183	DB:Swissprot Frame:2 orf:5 Homolog: Peroxiredoxin-5, mitochondrial Evaluate:3e-40 Bitscore:150		EZ048816	92	1	K. DLFFPIINDYHVGFDIEK. G (55) R. HLPSPYVK. K (10)	24%
C28	pre-mRNA binding K protein, hnRNP K [Xenopus laevis, Peptide, 396 aa]	gi 299029		58	1	K. INIEFDGTGVECSIADR. I (73) R. ILSISADIEITIGEILK. K (58)	4%
C42	Heterogeneous nuclear ribonucleoprotein K [Mus musculus]	gi 13384620		67	0	R. ITAVLSR. I (43)	7%
C47	PREDICTED: similar to aconitase, mitochondrial [Nasonia vitripennis]	gi 156537745		58	0	K. ILLLLLSGAK. I (24) K. NTIVTSYNR. N (25)	2%
C52	peptidase M17 precursor [Clonorchis sinensis]	gi 118429525		55	1	K. ILYSHLDEPQK. Q (33) K. GTTYDTGGADVK. A (55)	2%
C60	DB:Swissprot Frame:2 orf:1 Homolog:Gamma-glutamyltranspeptidase 1 Evaluate:6e-49 Bitscore:194		GH986789	53	2	K. DMSSPEQDLYHQ. R. F + Oxidation (M) (31) K. LKEFTSFQVAQSTR. R (22)	11%
C87	GDP-Mannose Dehydratase family member (gmd-2) [Caenorhabditis elegans]	gi 17507723		61	1	K. FYQASTSELYGK. V (61)	3%
C95	Short-chain dehydrogenase/reductase SDR YhdF [Bacillus licheniformis ATCC 14580]	gi 52079424		110	1	K. GAIVAFTR. S (51)	7%
C98	DB:Swissprot Frame:2 orf:1 Homolog:Uncharacterized oxidoreductase yhdF Evaluate:3e-28 Bitscore:125		(GH986692)	31	1	K. TAITGGDSSGIGR. A (59) K. TAITGASTGIGR. A (31)	6%
C98	DB:Swissprot Frame:3 orf:5 Homolog:Protein lethal(2)essential for life Evaluate:2e-11 Bitscore:709		EZ048820	59	2	R. GYRPEEVTLK. T (15) K. DGVLSVECPFLPQGNR. I (44)	30%
C98	DB:Swissprot Frame:-1 orf:1 Homolog:Probable transaldolase Evaluate:6e-34 Bitscore:144		GH986571	35	1	K. TVVMGASFR. N + Oxidation (M) (11) K. LLEELANSTAK. V (24)	18%

Table 2. Cont.

Spot no.	Protein name	NCBIhr Accession no. (')	Tardigrade specific Accession no. (contig/EST) (Δ)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
C110	aldolase A protein [Homo sapiens]	gi 28595		71	1	K. GLAADESTGSIAR. R (71)	12%
C111	DB:Swissprot Frame:2 orf:1 Homolog:Glycerinaldehyde-3-phosphate dehydrogenase Evalue:6e-77 Bitscore:2		GH986712	344	7	K. ADVKEQDQLSINGK. I (63) K. DVDVVAINDPFIDIK. Y (49) K. FGVIGLMTTVAFTATQK. V + Oxidation (M) (39) K. TMDIYSNASCTNCLAPLAK. V (77) R. AALDKDVVVAINDPFIDIK. Y (47) K. VIISAPSADAPMFVCGVNLDK. Y (33) K. VIISAPSADAPMFVCGVNLDKYDAK. T (35)	51%
C115	DB:Swissprot Frame:1 orf:6 Homolog: Plasminogen Evalue:6e-36 Bitscore:84.3		EZ048798	63	2	K. GDFDEFIR. I (34) R. AYSGGISADMLCGAAPGK. D (29) R. GCAQPNVPGVGR. M (46) K. DSCQGDGGGLVFLK. N (75) R. TTIFSPEGR. I (47)	19%
D159				121	2	R. AYSGGISADMLCGAAPGK. D (29) R. GCAQPNVPGVGR. M (46) K. DSCQGDGGGLVFLK. N (75) R. TTIFSPEGR. I (47)	21%
C126	DB:Swissprot Frame:2 orf:4 Homolog: Proteasome subunit alpha type-4 Evalue:4e-81 Bitscore:300		GH986859	47	1	R. TTIFSPEGR. I (47)	4%
C128	expressed hypothetical protein [Trichoplax adhaerens]	gi 196010133		105	1	K. VGSEATLLNMLK. V (105)	4%
	F25H2.10 [Caenorhabditis elegans] DEAD-box family (SNF2-like) helicase, putative	gi 17506815 gi 84996109		97 54	1 1	K. TSFFQALQIPFK. I (97) K. MLELISNLIK. K (54)	3% 0%
	[Theileria annulata] ResB family protein [ Hydrogenobaculum sp. Y04AAS1]	gi 195953863		54	1	K. MLELISNLIK. K (54)	1%
C132	DB:Swissprot Frame:1 orf:1 Homolog:Peroxiredoxin- 4 Evalue:4e-86 Bitscore:318		EZ048818	393	8	R. GLFIIDK. K (28) R. GLFIIDKK. G (32) K. TQIGKAPDFK. G (27) K. FENVLSDYK. G (57) R. QITMNDLPVGR. S (51) K. GKFENVLSDYK. G (59) R. CNVYGGDVPVPER. S (58)	31%

**Table 2.** Cont.

Spot no.	Protein name	NCBIr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
C139	DB:Swissprot Frame:1 orf:1 Homolog:ATP synthase		EZ048797	81	2	K. DYGVILEDAGHTLR. G (81) K. VLAFFESPAAK. I (33)	13%
D166	subunit d, mitochondrial	Value:4e-27 Bitscore:121				R. VPVFGIVDQFR. K (48)	
C143	Glyceraldehydes-3-phosphate dehydrogenase	gi 7274154		107	1	R. VPVFDVSVVDLTVR. I (107)	4%
C145^	[Achiya bisexualis]						
C143*	DB:Swissprot Frame:3 orf:2-4	gi 1351273	GH986530	281	5	K. AIADVISDMSK. V (67) R. EGNQTEVVFR. Q (47)	33%
B163^	Homolog:Triosephosphate isomerase B Value:4e-69 Bitscore:260					K. DVGAEWVILGHSER. R (82) K. VVIAYEFVWAIGTGK. T (47) K. EASGAFTEIISPAMLK. D (38)	
C145	DB:Swissprot Frame:2 orf:3 Homolog:Peroxiredoxin-		EZ048781	113	3	K. IGSPEAEDFK. A (41) R. GLFIIDQK. G (37) K. AVAVIDGQFQDIQLSTLK. G (35)	19%
C148	4 Value:4e-65 Bitscore:247 thiol-specific antioxidant protein [Homo sapiens]	gi 438069		54	1	R. QITVNDLPVGR. S	5%
C148	RecName: Full = Aspartic protease inhibitor 8	gi 124012		60	0	R. GALGGDYYLQK. S (12)	10%
C155	DB:Swissprot Frame:1 orf:1 Homolog:Superoxide dismutase [Cu-Zn] Value:2e-48 Bitscore:192		GH986811	401	6	R. GALGGDYYLQK. S (48) R. VTSAVAVMK. G (45) R. IACGIVGVGGTK. - (69) R. VTSAVAVMKGDSFVK. A + Oxidation (M) (32) R. GLPAAESKIHGNSGGR. I (70) R. HVGDLGNLVADASGTAQ. I (137) K. IDITDILMSLMGEHSIVGR. A + 2 Oxidation (M) (48)	33%
D13	DB:Swissprot Frame:1 orf:1 Homolog:40S ribosomal protein S12 Value:2e-34 Bitscore:144		GH986534	22	1	K. LDADSLPR. K (22)	6%
D53	PREDICTED: similar to alpha actinin CG4376-PB [Tribolium castaneum]	gi 91080533		120	1	R. YGWEQLLTSINR. N (47) R. NINEVENQILLTR. D (58)	3%



**Table 2. Cont.**

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	M/S/MS peptide sequence (Indv. Ion score)	Sequence coverage
D56	DBSwissprot Frame:1 orf:3 Homolog:Periostrin Evalue:1e-10 Bitscore:67.8	gi 148717319	EZ048782	22	1	K.QTBGEIVFIPDDAAAFGK.M(22)	6%
D57	protein disulfide isomerase-3 [Haemaphysalis longicornis]	gi 1169533		68	0	K.HGVYGYPTLK.I(48)	3%
D61	Endlase (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate hydro-lyase)	gi 1169533		94	1	R.GNFTVEVEVTDK.G(74)	6%
D109						K.VKIGMDVASSEFYK.D + Oxidation (M) (20)	
D61	DBSwissprot Frame:1 orf:1 Homolog:Matrix metalloproteinase-17 Evalue:2e-06 Bitscore:50.8		GH986535	17	1	R.FEVAEGFPK.S(17)	16%
D62	DBTrembl Frame:1 orf:1 Homolog: Vitellogenin 1		EZ048784	477	9	K.FGNNIGQNIIEK.Y(46)	34%
B19, C13, C15, C16, C28,	Evalue:2e-05 Bitscore:53.1					K.VLFDGNYVEIK.A(59)	
C33, D29, D30, D39),						K.KFGNNIGQNIIEK.Y(75)	
D42, D50, D58, E11, E12,						K.EPILAIIVSPVTGLK.V(74)	
E15, E45, E54, E65, E66,						R.AYLLQEGSCNAQIIPQDK.K(42)	
E73, F26						R.AYLLQEGSCNAQIIPQDK.V(36)	
						R.DELFAVLAANANPSASPLEIR.R(75)	
						K.VSEYTIYNGQPIPPPEGK.F(22)	
						-DNSRDELFAVLAANANPSASPLEIR.R(48)	
D81	DBSwissprot Frame:2 orf:1 Homolog:Actin-1 Evalue:6e-87 Bitscore:319		GH986913	33	1	K.EISALAPNTIK.-(33)	5%
D84	UspA [Bacillus coagulans 36D1]	gi 124521548		56	1	R.ILVAIDGSK.M(56)	6%
						K.ALYISQGI.R.A(38)	
	DBSwissprot Frame:3 orf:1 Homolog:Aldehyde dehydrogenase, mitochondrial Evalue:2e-47 Bitscore:188		EZ048791	160	4	K.YGLAASVMFK.D + Oxidation (M) (22)	37%
						-GYFIEFTFADVK.D(48)	
						R.ELGEYGLDAYTEVK.T(53)	
D90	DBSwissprot Frame:2 orf:1 Homolog:Annexin A6 Evalue:1e-37 Bitscore:143		EZ048803	223	6	K.DLFDLKK.E(24)	48%
D89, D96						R.DHYNPTIR.A(21)	
						K.GIGTDEDIVTK.I(44)	

Table 2. Cont.

Spot no.	Protein name	NCBIhr Accession no. (*)	Tardigrade specific Accession no. (contig/EST) (^)	Total protein score	No. of unique/ significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
						R. HLLFAITTR. R (47)	
						R. AFQPFNDNDK. A (38)	
						R. EVIDDIVSDTSGYFR. H (43)	
D91*	elongation factor 1 gamma [Bombyx mori]	gi 112983898	EZ048793	60	1	K. ATAGAGTSEEDLIEIMLTR. N + Oxidation (M) (6) K. VPAFESADGK. V (58)	2%
D104°, D106°							
D92	mitochondrial long-chain enoyl-CoA hydratase/3-	gi 510108		57	0	K. ALTSFER. D (7)	3%
	hydroxyl-CoA dehydrogenase alpha-subunit					K. DGGFYTR. C (34)	
	[Rattus n					K. VIGMHYFSFVDK. M (16)	
	DB:Swissprot Frame:3 orf:1 Homolog: Peroxidase		EZ048773	30	1	R. TGETTDQMAILK. K + Oxidation (M) (30)	7%
D96	Value:7e-19 BitScore:94.0 cysteine conjugate beta-lyase [Takifugu rubripes]	gi 5002565		84	1	K. ALVINTPNNFLGK. V (84)	3%
D96	DB:Swissprot Frame:3 orf:5 Homolog:265 proteasome non-ATPase regulatory subunit 13		GH986860	100	3	K. LLEVEK. K (12) K. KLLLEVEK. K (26)	15%
	Value:1e-29 Bit					R. SAGMSELYK. N (32)	
						R. LHGTAEYFR. E (31)	
D103	DB:Swissprot Frame:1 orf:1 Homolog:Nephrilysin-2 Value:9e-31 BitScore:133		EZ048772	32	1	K. IIAQYSNFR. Y (32)	6%
D107	Tubulin alpha-3 chain (Alpha-III tubulin)	gi 3915094		252	2	R. LSVDYK. K (27)	18%
						R. QLFHPEQLITGK. E (31)	
						R. IIGQIVSSITASLR. F (29)	
						R. AVFDLEPTVIDEIR. T (64)	
						R. NLDIERPTTNLR. L (81)	
						R. FDGALNVDLTFQTNLVYFR. I (21)	
D110	Tubulin beta-3 chain (Beta-tubulin class-IV)	gi 135464		152	1	R. FPGQLNADLR. K (70)	7%
						K. LAVNVPFPR. L (36)	

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indiv. Ion score)	Sequence coverage
D111	GH19645 [Drosophila grimshawi]	gill195053606		120	2	R.AVLVDLEPCTMDSYR.S (46)	7%
						K.KGIDAEVINLR.S (56)	
D118	DB:Swissprot Frame:1 orf:4 Homolog:Histone H4	GH986770		18	1	R.VFLGEEVAQYDGAYK.V (64)	12%
						R.ISGLIYEETR.G (18)	
	Value:9e-39 Bitscore:160			15	1	K.LLLPGLAK.H (15)	9%
						DB:Swissprot Frame:3 orf:1 Homolog:Histone	
D140	H2B.2 Value:8e-32 Bitscore:135		EZ048771	381	7	K.LGSWTFPK.D (51)	23%
						DB:Swissprot Frame:2 orf:3 Homolog:Acetylcholine receptor subunit alpha-L1 Value:1e-16 Bitscore:87	
D128						R.LQYTDNAVYK.K (34)	
						R.LQYTDNAVYK.I (34)	
						K.DELDVQTSQSK.F (68)	
						R.AFLSLNWDHR.L (80)	
						K.FDDYFQSSVWK.F (61)	
						K.LGSWTFPKDELVDVQTSQSK.F (53)	
D159	DB:Swissprot Frame:2 orf:1 Homolog:Peroxiredoxin-6 Value:8e-61 Bitscore:233	GH986904		403	8	K.LAPEFEK.R (38)	53%
						R.NFDLRLR.V (27)	
						R.VLDSLQIVSK.H (63)	
						K.HSVVTFVDWK.- (69)	
						K.LVLIYFATSGR.N (50)	
						K.DLESYCGMGKK.F + Oxidation (M) (48)	
						K.MIALSCDDAQSHQGWIK.D + Oxidation (M) (40)	
						K.FGMLDFEELNINNNPVTAR.A + Oxidation (M) (68)	
E4	phosphoglycerate kinase[Verrucomicrobiae bacterium V4]	gill161075769		54	1	K.AIGFLMEKELK.Y + Oxidation (M) (54)	2%
E5	Rubber elongation factor protein (REF) (Allergen)	gill132270		104	1	R.SLASSLPQGTK.I (33)	18%
D99	Hev b 1)					K.FVDSTVVASVTIDR.S (71)	
E5	Small rubber particle protein (SRPP) (22 kDa rubber particle protein) (22 kDa RPP) (Latex allergen)	gill14423933		87	0	K.AEQYAVITWR.A (43)	14%
						R.IVLDVASSVFNTGVQEGAK.A (44)	

**Table 2.** Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
	DB:Swissprot Frame:2 orf:1 Homolog:Liver carboxylesterase Evalue:2e-33 Bitscore:142		EZ048809	21	1	K. AIVAVVNR. V (21)	5%
E43*	actin [Heliolithis virescens]	gi 14010639	(EZ048826)	667	7	K. EITALAPSTMK. I (41) R. AVFPSIVGRPR. H (73) K. IMHHTFYNELR. V (73) K. QEYDESGPSIVHR. K (94) K. SYELPDGQVITIGNER. F (79) R. VAPFEEHFVLLTEAPLNPK. A (90) K. YPIEHGIIINWDDMEK. I (56) K. DLYANTVLSGGTMYPGIADR. M (45) R. KDLYANTVLSGGTMYPGIADR. M+Oxidation (M) (36) R. TTGIVLDSGDGVSHVPIYEGYALPHAILR. L (55) R. DLTDYLMK. I (25)	41%
E43*	DB:Swissprot Frame:1 orf2 Homolog: Actin-5C Evalue:7e-155 Bitscore:547		EZ048826	471	9	R. DLTDYLMK. I (25) R. GYSFVTTAER. E (38) K. EITALAPSTMK. I (41) K. AEYDESGPSIVHR. K (112) K. SYELPDGQVITIGNER. F (79) K. DLYANTVLSGGTMYPGIADR. M (45) R. KDLYANTVLSGGTMYPGIADR. M+Oxidation (M) (36) K. LCVVALDFEEMATAMASSLEK. S (39) R. TTGIVLDSGDGVSHVPIYEGYALPHAILR. L (55) K. RGIITLK. Y (23) K. AGFAGDDAPR. A (62) R. DLTDYLMK. I (24) R. GYSFVTTAER. E (40) K. EITALAPSTMK. I + Oxidation (M) (40) R. AVFPSIVGRPR. H (59) K. IMHHTFYNELR. V (60) K. QEYDESGPSIVHR. K (58) K. SYELPDGQVITIGNER. F (79) R. VAPFEEHFVLLTEAPLNPK. A (75)	37%
D99, D106, D108, E71, E72, E84, E92, E94, E99, E100, F44, F58, F61, F95							
E47*	Actin, muscle-type (A2)	gi 3121741	(EZ048826)	519	6	K. RGIITLK. Y (23) K. AGFAGDDAPR. A (62) R. DLTDYLMK. I (24) R. GYSFVTTAER. E (40) K. EITALAPSTMK. I + Oxidation (M) (40) R. AVFPSIVGRPR. H (59) K. IMHHTFYNELR. V (60) K. QEYDESGPSIVHR. K (58) K. SYELPDGQVITIGNER. F (79) R. VAPFEEHFVLLTEAPLNPK. A (75)	30%
F64*, F95*							

**Table 2.** Cont.

Spot no.	Protein name	NCBI nr Accession no. (')	Tardigrade specific Accession no. (contig/EST) (v)	Total protein score	No. of unique/ significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
E48*	actin 5C [Lycosa singoriensis]	gi 161661023	(EZ048826)	644	6	K.AGFAGDAPR.A(80)	35%
E44*						R.DLTDYIMK.I + Oxidation (M) (15)	
						R.GYSFVTTAER.E(44)	
						R.AVFPISIVGRPR.H(98)	
						K.IWHHTFYNELR.V(66)	
						K.QEYDESGFSIVHR.K(100)	
						K.SYELPDGQVITIGNER.F(70)	
						R.VAPEEHPVLLTEAPFLNPK.A(83)	
						K.YPIEHGIITNDDMEK.I + Oxidation (M) (25)	
						K.DLYANTVLSGGTMYFGIADR.M(47)	
E50*	beta-actin [Rachycentron canadum]	gi 161376754	(EZ048826)	501	4	R.KDIYANTVLSGGTMYFGIADR.M+Oxidation (M) (18)	33%
E6*, E42*, E49*, F27*						K.AGFAGDAPR.A(59)	
F28*						K.EITALAPSTMK.I(49)	
						R.AVFPISIVGRPR.H(73)	
						K.IWHHTFYNELR.V(46)	
						K.SYELPDGQVITIGNER.F(79)	
						M.EEEIAALVVDNGSGMCK.A(50)	
						R.VAPEEHPVLLTEAPFLNPK.A(43)	
						K.DLYANTVLSGGTMYFGIADR.M(24)	
E52*	alpha-actin (aa 40-375) [Mus musculus]	gi 49864	(EZ048826)	106	0	R.KDIYANTVLSGGTMYFGIADR.M+Oxidation (M) (53)	11%
E85*, D103*						R.GYSFVTTAER.E(34)	
						K.EITALAPSTMK.I + Oxidation (M) (22)	
E57	muscle actin	gi 797290		290	1	K.SYELPDGQVITIGNER.F(50)	25%
D104*, D97*, E59*, E68*, E67*, E59*, E58*, E55*, E53*, E93*						R.DLTDYIMK.I(24)	
						R.GYSFVTTAER.E(43)	
						K.EITALAPSTMK.I(49)	
						K.IWHHTFYNELR.V(39)	
						K.QEYDESGFSIVHR.K(58)	
						R.VAPEEHPVLLTEAPFLNPK.A(39)	
E63	DB:Swissprot Frame:1 orf:1 Homolog:AFG3-like protein 2 Evalue:9e-58 Bitscore:221		GH986706	35	1	K.YPIEHGIITNDDMEK.I(19)	12%
						K.CFELLSEK.K(9)	
						K.GLGYAQLPR.E(27)	

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (°)	Tardigrade specific Accession no. (contig/EST) (△)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
E64	heat shock protein 90 alpha [Fundulus heteroclitus macrolepidotus]	gi 77999578		62	1	R.FTSSASGDEMVSLLK.D+ Oxidation (M) (62)	6%
E70*	actin [Paraphidippus auranitus]	gi 167683068	(EZ048826)	376	2	R.DLFDYIMK.I (15) R.GYSFVTTAER.E (41) K.EITALAFSTMK.I (46) K.IWHHTFYNELR.V (40) K.SYELEDGQVITIGNER.F (67) R.VAPEHFVLLTEAPINPK.A (59) K.YPIEHGIIITWDDMEK.I (8)	42%
E73	PREDICTED: similar to Nucleosome remodelling factor - 38kD CG4634-PA [Apis mellifera]	gi 66507623		85	1	R.TTGIVLDSGGVSHVPIYEGYALPHAILR.L (16) K.GDNDFIDVLEIGYK.V (85)	1%
E74	DB:Swissprot Frame:1 orf:4 Homolog: Protein	GH986548		118	3	K.SLAPEYAK.A (16) K.DNFEDALK.E (21) K.VDAIVETDIAATK.Y (80)	17%
E76, E88, F62	disulfide-isomerase					K.VGDFGPFISGR.E (47)	11%
E80	DB:Swissprot Frame:1 orf:3-5 Homolog: Cathepsin Z	GH986945		102	2	K.TFNQCGTCSEFGK.C (55) K.DLIPDSSLR.T (40)	23%
E83	proteasome non-ATPase regulatory subunit 8	EZ048799		154	4	R.IYYDWK.D (5) K.CEALLNQIK.V (21) R.DVLEMGQQLAALK.R (45) R.ACPEVNLNSLCR.M (44) K.LSSAHVYLR.L (21)	6%
E86	DB:Swissprot Frame:3 orf:4 Homolog: Coiled-coil domain-containing protein 25	EZ048808		21	1		6%
E89	ATP synthase beta subunit [Asteria miniata]	gi 46909233		346	3	K.AHGYSVTFAGVGER.T (33) R.FTQAGSEVSALLGR.I (97) R.VALTGLTVAEYFR.D (84) K.TVLIIMELINNVAK.A (62) K.VALVYGMNEPFGAR.A + Oxidation (M) (38)	24%
D140							

Table 2. Cont.

Spot no.	Protein name	NCBIhr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (indv. ion score)	Sequence coverage
						R. GIAELGIYPAVDFDSTSR. I (26)	
E46				191	0	R. EGNDIYHEMIEGGVISLK. D + Oxidation (M) (7) K. IGLFGAGVGK. T (41) R. IIVGFETLGR. I (34) K. VVDLLAPYAK. G (40)	13%
						R. TIAMDGTEGLIR. G + Oxidation (M) (44) R. FTQAGSEVSALLGR. I (33) K. VALELLGFIR. Q (54)	
E89	DB:Swissprot Frame:2 orf1 Homolog:rab GDP dissociation inhibitor beta Evalue:4e-51 Bitscore:201		GH986887	152	3	R. GTGQVDFETK. V (55) R. CICLLDHPINPK. D (6) K. DALSTQIIIPQNVNR. N (33) R. NNDIYISVVSYTHVAAK. G (4) K. TVTSLWR. E (22) R. ASFGADNMFYFK. A (54)	45%
						R. TACTAEIGIDK. V (50)	
E92	DB:Swissprot Frame:1 orf1 Homolog:Methylmalonate-semialdehyde dehydrogenase [acylating], mitochond		GH986892	75	2		11%
E93							
E98	DB:Swissprot Frame:3 orf1 Homolog:Thioredoxin reductase 1, cytoplasmic Evalue:2e-73 Bitscore:275		GH986518	50	1		5%
						R. IDSEFTIK. I (43)	
F3	DB:Swissprot Frame:2 orf1 Homolog:Protein disulfide-isomerase 2 Evalue:3e-64 Bitscore:244		EZ048794	527	9	R. ITEFFGLTK. D (57) K. NFEDEVMDK. S (56) R. ILSLADQLVK. Y (46) K. GDNVVEYGER. T (38) K. MDAFANELEHTR. I + Oxidation (M) (72) K. KGDNTVVEYGER. T (62) K. LSPFYDELGDHFK. D (79) K. YKFEAGDLNPETLTK. F (64) K. LKPHLNSQVDFEDWNAK. S (10) R. YLTVAAIFR. G (15)	56%
F1, F2, F27, F43, F49							
F6	Tubulin beta-1 chain (Beta-tubulin class-I)	gi 57429		54	0		4%

Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
F12	hsp 108 [Gallus gallus]	gi 63509		84	0	R.FPQLNADLR.K(39) R.ELIASNASDALDK.I(50) K.GVVSDDDLPLNVS.R.E(31) R.ELIASNASDALDK.I(77) K.FVWTACK.F(23) K.FYGDADLNK.G(42) K.VFFSTMDQK.D(48) R.FYGLSAAFFK.F(41) K.DKPLIVQFSVK.H(59) K.HEQIDCGGYVK.V(88) K.EQFLDNKWE.DR.W(65) R.EIENPAYK.G(19) K.AAEDFANDTWGK.T(85) K.SGTIFDDIIITDDIK.A(54) R.LSPEDIER.M(39) K.FDLTGLFPAPR.G(43) K.FEELNNDLFR.A(47) K.HFSVEGQLEFR.A(42) K.EKYIDQBELNK.T(16) K.ADLMNNLGTIAK.S + Oxidation (M) (62) R.YMSLTDPK.Q + Oxidation (M) (6)	4%
F13	hsp 82 [Drosophila pseudoobscura]	gi 9069		77	1		3%
F14*	DB:Swissprot Frame:3 orf:2 Homolog:Calreticulin	gi 195107681	GH986835	365	7		40%
B195^, B198^	Evalue:2e-69 Bitscore:262						
F38	DB:Swissprot Frame:2 orf:1 Homolog:Calreticulin		GH986920	159	3		47%
F21	Heat Shock Protein family member (hsp-3)	gi 17568549		180	0		6%
F22, F23	[Caenorhabditis elegans]						
F24	heat shock protein 90-beta [Danio rerio]	gi 18858875		83	0		5%
F24	heat shock protein 90 [Danio rerio]	gi 555574	EZ048788	62	1		11%
F24	DB:Swissprot Frame:2 orf:3 Homolog:Heat shock			214	3		21%
F25	protein 83 Evalue:1e-84 Bitscore:312						
F25	Heat shock protein HSP 90-alpha	gi 17865490		275	1		8%
F27	heat shock protein 70 [Lirionyx huidobrensis]	gi 89892741		225	3		4%



Table 2. Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/ significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
	Heat Shock Protein family member (hsp-1) [Caenorhabditis elegans]	gjl17541098		219	2	K. MDRKQIHDIVYGGSTR. I + Oxidation (M) (31) R. LSKDDIER. M (52) R. FEELCADLFR. S (68) R. ARFEELCADLFR. S (58)	6%
<b>F32</b>	D8:Trembl Frame:1 orf3 Homolog:Lipoprotein- related protein Evalue:7e-07 Bitscore:56.2		GH986605	19	1	K. SINPDEAVYGAOVAAIISGDK. S (41) K. VIASIDLTGK. T (19)	9%
<b>F35*</b> <b>F36*, F53*</b>	tropomyosin	gjl42559676		273	2	K. IVELEELR. V (55) K. LAMVEADLER. A (44) R. EDSYEEQIR. L (74) R. KLAMVEADLER. A (51) K. ALQREDSYEEQIR. L (18) R. IQLLEDDL. T (69)	17%
	D8:Swissprot Frame:1 orf2 Homolog:Tropomyosin- 1, isoforms 9A/A/B Evalue:3e-30 Bitscore:131		GH986919	229	3	K. LSEASQADESER. A (93) R. IQLLEDDLTERTEER. L (67) R. LEDDLVHEK. E (35)	18%
	D8:Swissprot Frame:2 orf1 Homolog:Tropomyosin Evalue:3e-11 Bitscore:66.6		GH986674	48	1	K. EVDREDDLVHEK. E (13)	38%
<b>F36</b> <b>F33, F37, F64,</b> <b>F69, F71,</b> <b>F72</b>	D8:Trembl Frame:2 orf1 Homolog:Putative uncharacterized protein Evalue:1e-18 Bitscore:97.1		EZ048810	109	2	R. DYPIYNDIPSTR. F (51) K. QAGFYADAEEAQQQVIR. R (58)	28%
<b>F39</b>	D8:Trembl Frame:2 orf3 Homolog: CG14304-PA		EZ048802	104	2	K. DYPTVNEIPNTR. F (55)	32%
<b>F34, F42, E64, E90</b>	Evalue:9e-18 Bitscore:95.5					K. QAGFYADIDAQQQAIR. R (49)	
<b>F42</b>	D8:Swissprot Frame:2 orf1 Homolog:Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial E		GH986609	53	2	K. EEQVQEAFR. I (35) K. LPIIAANSLIEDAAAK. A (19)	17%
<b>F51</b> <b>E60, E63, E96, F31,</b> <b>F32,</b>	D8:Trembl Frame:2 orf1 Homolog:AGAP009479- PA Evalue:7e-15 Bitscore:83.2		EZ048819	58	1	K. QAGFYADTDAQQQVIR. R (58)	16%

Table 2. Cont.

Spot no.	Protein name	NCBIr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
F52, F70, F73, F96							
F54	DB:Trembl Frame:1 orf:1 Homolog:Putative uncharacterized protein		EZ048790	62	1	K.QAGFYADTEAQQVIR.R (62)	18%
F40, F41, F66, F67, F68	Value:2e-16 Bitscore:88.6						
F55	DB:Swissprot Frame:2 orf:1 Homolog:CD109		EZ048800	29	1	R.TVVVYDYINPQDR.K (29)	20%
F56	antigen Value:2e-06 Bitscore:52.4 GTP-specific succinyl-CoA synthetase beta subunit	gi 4406564		59	1	K.INFDDNAEFR.Q (59)	5%
F57	[Homo sapiens] heat shock protein 60 [Salmo salar]	gi 16923167		63	1	K.VGGTSEVYNEK.K (63)	7%
F65*	14-3-3 protein beta/alpha-2 (Protein 14-3-3B2) [Onchorynchus mykiss]	gi 82089139	(GH986681)	90	0	R.NLLSVAYK.N (48)	6%
F73	DB:Swissprot Frame:2 orf:2 Homolog:60S ribosomal protein L26-1		GH986676	21	1	R.YDDMAGAMK.S (42) K.LSKDVSSSR.R (21)	6%
F75	Value:6e-45 Bitscore:179 DB:Swissprot Frame:2 orf:2 Homolog:Translationally-controlled tumor protein		EZ048806	234	3	R.IVEVPELQDK.K (56) K.IVDNVLFVYTK.Y (86) K.DAVTGDMEFSDSYK.Y (80)	33%
F81	homolog Value:1e-57 Bi						
F80, F89	DB:Swissprot Frame:2 orf:4 Homolog:Myosin regulatory light polypeptide 9		EZ048792	331	6	K.RVQEQSPNEVDQFK.T (8) K.DTFASLGR.A (30) R.DLLGGVGDK.L (57) K.GOLDYVAFAK.I (42)	37%
F81	Value:4e-50 Bitscore:198						
F81	DB:Swissprot Frame:2 orf:1 Homolog:Cathepsin L1		GH986678	98	1	K.LSADEMSQAFK.G + Oxidation (M) (67) K.EAFTMDQNR.D + Oxidation (M) (38) K.VAGYDFEATITNAFK.I (97) K.LPLDSEQNIVDCSK.K (98)	5%
F87	Value:2e-65 Bitscore:249 DB:Swissprot Frame:3 orf:1 Homolog:Troponin C		GH986791	24	1	R.QIGTLIR.T (24)	4%
F88	Value:3e-60 Bitscore:231 putative LEA III protein isoform 2 [Corylus avellana]	gi 14148981		69	1	K.AGESQYQDTANAAK.N (69)	16%

**Table 2.** Cont.

Spot no.	Protein name	NCBI nr Accession no. (1)	Tardigrade specific Accession no. (contig/EST) (2)	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage
	glycosyl transferase, family 2 (Shewanella sediminis HAW-EB3)	gjl157373461		59	1	R.HLLISLADK.L (59)	1%
	DB:Swissprot Frame:2 orf:5 Homolog: Myosin, essential light chain Evalue:8e-30 Bitscore:131		EZ048813	404	10	K.EVDEILR.L (20) R.HLLLSLGEK.L (59) K.ESNGTIIAAELR.H (68) K.DVGTLEDFFMEAMR.V (73) K.LTVEEFMPYGLSK.E (28) R.VFDKESNGTIIAAELR.H (26) K.EKDVGTLDFMEAMR.V (23) K.EVFGVYDMFFGGTINK.V (45) K.KLTFVEEFMPYGLSK.E (26) K.EVFGVYDMFFGGTINKVDAMK.V (37) K.NTTCEFTGDIILR.T (52) R.TVSGVNGPIVITIDDDVK.F (51)	58%
<b>F95</b>	SICHGC06651 protein [Schistosoma japonicum]	gjl56759014		103	0		9%

Generated MS/MS data were searched against the NCBI nr and tardigrade protein databases. Spot number, protein annotation, accession number, total protein score, number of matched peptides, peptide sequence and sequence coverage are listed. Identical proteins identified in different spots are listed only once and the spot with the highest protein score (in bold) is ranked at the top.  
doi:10.1371/journal.pone.0009502.t002

**Table 3.** Identified proteins without annotation.

Spot no.	Accession no.	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage	DomainSweep analysis
A11	GH986700	52	1	- .VIAVSLPR.N (52)	3%	No hits
A82, A88, B33, B41, B43, C50, D99, D105, E72, F87						
A11	GH986755	32	1	- .LSISHNATLR.V (32)	4%	Putative IPR006210EGF
A94	GH986643	39	1	R.VDRSIPR.L (39)	3%	Putative IPR004077 Interleukin-1 receptor, type II
A91, A95, A110, A123, A140, B49, B64, B83, B90, B98, B105, B155, B165, B173, B176, B185, B186, B187, B188, B189, B190, B191, B192, B193, B194, B195, C51, C128, C141, C153, D45, D46, D56, D57, D74, D123						
A100	EZ048767	229	4	K.YDLIYK.G (15)	20%	Putative IPR017956 AT hook, DNA-binding, conserved site
K.FLGFDTAGK.T (61)						
K.IISFDVCNK.N (54)						
K.TDSGVSCDVTD-KCDPIVK.A (39)						
K.AVVDIEDPNN-SAGDSIDYK.Y (60)						
A112	GH986667	317	5	R.EQFTQGCTVGR.N (61)	22%	Putative IPR001749 GPCR, family 2, gastric inhibitory polypeptide receptor
A114				K.LEAAPNQCPEYK.K (89)		IPR000372 Leucine-rich repeat, cysteine-rich flanking region, N-terminal
K.KLEAAPNQCPEYK.K (64)						
K.IMEVCNEPNTYENVNR.F + Oxidation (M) (44)						
K.IQSLCTPADLQ-FFQSTHDR.I (60)						
A112	EZ048821	98	2	K.NADPLTILK.E (37)	14%	Putative IPR004825 Insulin/IGF/relaxin IPR008355 Interferon-gamma receptor alpha subunit
K.IQSLCTPADLQ-FFQSTHDR.I (60)						
A114	EZ048817	49	1	R.IGTETTSFDYLR.E (49)	3%	Putative IPR004354 Meiotic recombination protein rec114
A123	EZ048785	221	4	K.FLDFTR.G (28)	17%	Putative IPR000762 PTN/MK heparin-binding protein
R.AADLDTLTK.L (57)						
R.YLDMQYDW-DTR.S + Oxidation (M) (54)						
R.GTFDTAHIQG-LTALTTLR.L (60)						

Table 3. Cont.

Spot no.	Accession no.	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage	DomainSweep analysis
A136	EZ048814	55	1	R. IMSVDLTDINS-APGMFDAAK.T + 2 - Oxidation (M) (23) R. IPAQFQSK.I (55)	5%	Putative IPR015874 4-disulphide core
B48	EZ048766	273	5	K. QVNAETFQK.A (36) K. YSETVHYEGGK.Q (39) R. VDYVYSYHTK.M (4) R. GDFWSTDKPHR.Y (32) K. YDIALDTVEATLK.S (70) R. LIPDELLGTYEFSGK.Q (93)	24%	Putative IPR000507 Adrenergic receptor, beta 1 IPR000463 Cytosolic fatty-acid binding IPR004825 Insulin/IGF/relaxin
B61	GH986621	231	6	R. VLNNGVLR.V (39) R. VITVPEGIK.V (49) R. SLLGEIPITK.G (38) R. RVITVPEGIK.V (46) R. VITVPEGIKVESFK.S (26) K. GSLTAGSSSNTSGST-GSSSYSSGTTGSSGTSGGK.T (34)	13%	Putative IPR001610 PAC motif (peptide matched in frame 4) IPR007758 Nucleoporin, Nsp1-like, C-terminal (peptide matched in frame 6)
B62	EZ048776	230	6	R. VLNNGVLR.V (39) R. VITVPEGIK.V (49) R. SLLGEIPITK.G (38) R. RVITVPEGIK.V (46) R. VEAPIQVDQLTADQQR.S (93) R. VLNNGVLRVEAPIQVDQLTADQQR.S (69)	18%	Putative IPR007758 Nucleoporin, Nsp1-like, C-terminal
B79	GH986933	38	1	K. NGDVSIPIR.Q (38)	6%	No hits
B91	GH986939	54	1	R. EALSAVTGGR.R (62)	9%	No hits
B102	EZ048815	403	6	K. QVNAETFNK.A (40) K. GGPAPWPKDEK.F (17) K. ILFRPTLSAR.A (36)	26%	Putative IPR000507 Adrenergic receptor, beta 1 IPR006080 Mammalian defensin

Table 3. Cont.

Spot no.	Accession no.	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage	DomainSweep analysis
B110, B111, B144				R. AQGLWEATTEGK. N (68)		IPR002181 Fibrinogen, alpha/beta/gamma chain, C-terminal globular
				R. LIPDELLGTFEFSGK. Q (92)		IPR000463 Cytosolic fatty-acid binding
				R. RLIPDELLGTFEFSGK. Q (36)		
				K. DYEFKEDGNMQMTAK. F + Oxidation (M) (20)		
				K. EVEYTSNYDMALDTVK. A (51)		
				R. MGLGVWESTSEQ ENMLEYLK. A (22)		
				R. GDKPGLAAF GDNIIEYSFTA-DSEGETGVLHGK. F (21)		
B103	EZ048768	40	1	R. VITVSIPR. I (40)	3%	No hits
B185, C150, C151, C153						
B150	GH986581	108	3	R. VFVEEQLK. A (33)	14%	Putative
B151, B173				R. FNFLVFLGSTR. E (46)		IPR000990 Innexin
				R. GHTYEIMDPEK. V + Oxidation (M) (29)		
B152	EZ048775	42	1	R. KLEFILXFIF. - (42)	5%	Putative
						IPR003061 Colicin E1 (microcin) immunity Protein
						IPR000048 IQ calmodulin-binding region
B179	GH986603	53	1	R. AFEVPASECGK. S (53)	5%	Putative
						PR015880 Zinc finger, C2H2-like
						IPR008264 Beta-glucanase
B191	EZ048789	26	1	K. GSIGAPDVPK. N (26)	4%	Putative
						IPR001955 Pancreatic hormone
B186	GH986708	468	6	R. AFEVPASECGK. S (46)	25%	Putative
A140				R. AFEVPASECGKSPK. R (82)		IPR015880 Zinc finger, C2H2-like
				R. YRAFEVPASECGK. S (36)		IPR000436 Sushi/SCR/CCP
				K. IVSKDVCSSPKPR. K (90)		IPR008264 Beta-glucanase
				R. SESGALWSEEQECTAK. F (62)		IPR000008 C2 calcium-dependent
				R. SESGALWSEEQ ECTAKFHR. D (137)		membrane targeting
				R. VQVMDKDVGSSDDLVEQ-FECLTGFLVSSR. S+Oxidation (15)		
C18	EZ048777	46	1	R. NLADQAMSGDGPLNFAK. A + 2 Oxidation (M)	8%	Putative
						IPR003569 Cytochrome c-type biogenesis
						Protein Ccb5
						IPR002282 Platelet-activating factor

Table 3. Cont.

Spot no.	Accession no.	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage	DomainSweep analysis
						receptor
C78	GH986847	32	1	K. SEVFPRIR. S (32)	3%	Putative
B188, B173, C141						IPR003916 NADH-ubiquinone oxidoreductase, chain 5
C86	GH986916	196	4	K. NPYLELTPDK. - (38)	12%	Putative
				K. TPEESEAPQAIR. R (68)		IPR000863 Sulfotransferase
				K. TPEESEAPQAIRR. K (58)		IPR003504 Glial cell line-derived neurotrophic factor receptor alpha 2
				K. VEKTPEESEAPQAIR. R (32)		neurotrophic factor receptor alpha 2
C95	GH986921	35	1	- . VIAVSLPR. N (30)	2%	No hits
B18, B19, B47, B49, B138, C51, C62, C65, D107						
C95	GH986692	31	1	K. TALITGASTGIGR. A (31)	5%	Significant
						IPR002347 Glucose/ribitol dehydrogenase
						IPR002198 Short-chain dehydrogenase/reductase SDR
						Putative
						IPR003560 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase
						IPR002225 3-beta hydroxysteroid dehydrogenase/isomerase
C110	GH986711	31	1	K. ERSPLANK. I (31)	4%	Putative
						IPR006210 EGF
C118	EZ048824	45	0	K. DSVAIQFPK. D (24)	7%	Putative
				K. ADEAGFTDAIK. A (21)		IPR003535 Intimin bacterial adhesion mediator protein
C141	EZ048801	395	6	R. NQVYQSMER. H (34)	22%	Putative
C117, C145				R. QNIDAIEIPR. L (78)		IPR002546 Myogenic basic muscle-specific protein
				K. DFLSAVVNSIQR. R (58)		specific protein
				R. LSQLAVDSVEIAK. D (74)		IPR000795 Protein synthesis factor, GTP-binding
				R. MTISEPFESAEALK. D + Oxidation (M) (72)		binding
				R. LEDVDDVLMFAFGMLK. A + 2 Oxidation (M) (26)		
				R. MTISEPFESAEALKDMIVR. L + 2 Oxidation (M) (15)		
				R. LQSSPTLSSL VDQDTFELIR. Q (37)		
C141	GH986597	27	1	- . TAVEAVVR. T (27)	4%	Putative
						IPR003065 Invasion protein B
C156	EZ048804	277	5	K. QFPFPIQSAK. H (43)	27%	Putative

Table 3. Cont.

Spot no.	Accession no.	Total protein score	No. of unique/significant peptides	MS/MS peptide sequence (Indv. Ion score)	Sequence coverage	DomainSweep analysis
				R. NELGAQYNFK. I (44)		IPR001610 PAC motif
				R. VIQAATEILPGK. - (73)		IPR001713 Proteinase inhibitor
				K. LGHFQQYDVR. L (60)		IPR000010 Proteinase inhibitor I25,
				K. DRNELGAQYNFK. I (52)		cystatin
				K. HTGGSDFLI ADPEAQGVADAVR. S (4)		IPR001878 Zinc finger, CCHC-type
<b>D87</b>	<b>GH986563</b>	<b>35</b>	<b>1</b>	<b>K. DNVPLFVGR. V (35)</b>	<b>4%</b>	<b>Putative</b>
						IPR000215 Protease inhibitor I4, serpin
<b>D110</b>	<b>EZ048786</b>	<b>46</b>	<b>1</b>	<b>R. FATPLLLTGSK. D (3)</b>	<b>6%</b>	<b>Putative</b>
				<b>R. DVSPHPAACLTHSGR. V (43)</b>		<b>IPR002353 Type II antifreeze protein</b>
						<b>IPR002371 Flagellar hook-associated protein</b>
						<b>IPR000204 Orexin receptor</b>
<b>E9</b>	<b>GH986691</b>	<b>257</b>	<b>7</b>	<b>K. YANPQELR. Q (51)</b>	<b>31%</b>	<b>Putative</b>
D2-D5, D8, D18, D10, D13,				<b>K. SINVPQVEK. E (32)</b>		<b>IPR000980 SH2 motif</b>
D14, D15, D19-D23, D27, D28,				<b>K. QYWPYVDEKPR. M (46)</b>		<b>IPR000463 Cytosolic fatty-acid binding</b>
D31, D40, D47, E3, E4, E6, E7,				<b>K. KQYWPYVDEKPR. M (30)</b>		
E8, E10, E11, E12, E14, E15,				<b>R. DEDSFLYETPEA QNPIVQK. K (28)</b>		
E16, E18, E19, E60, E61, E63,				<b>K. RDEDSFLY ETPEAQNPVQK. K (37)</b>		
E64, F31, F94, F95				<b>K. GLESETEDTAATTILIADMVHY- LK. Y (33)</b>		
<b>F6,</b>	<b>GH986624</b>	<b>35</b>	<b>1</b>	<b>R. ESLDFFR. V (35)</b>	<b>3%</b>	<b>No hits</b>
<b>F48</b>						
<b>F63</b>	<b>GH986878</b>	<b>38</b>	<b>1</b>	<b>K. AEETVPVLLTAEK. L (38)</b>	<b>7%</b>	<b>Significant</b>
						<b>IPR007327 Tumor protein D52</b>
						<b>Putative</b>
						<b>IPR004077 Interleukin-1 receptor, type II</b>

Generated MS/MS data were searched against the tardigrade clustered database. Spot number, protein annotation, accession number, total protein score, number of matched peptides, peptide sequence and sequence coverage are listed. Identical proteins identified in different spots are listed only once and the spot with the highest protein score (in bold) is ranked at the top. The significant or putative candidates found in Domain Sweep are also listed in the Table.  
doi:10.1371/journal.pone.0009502.t003

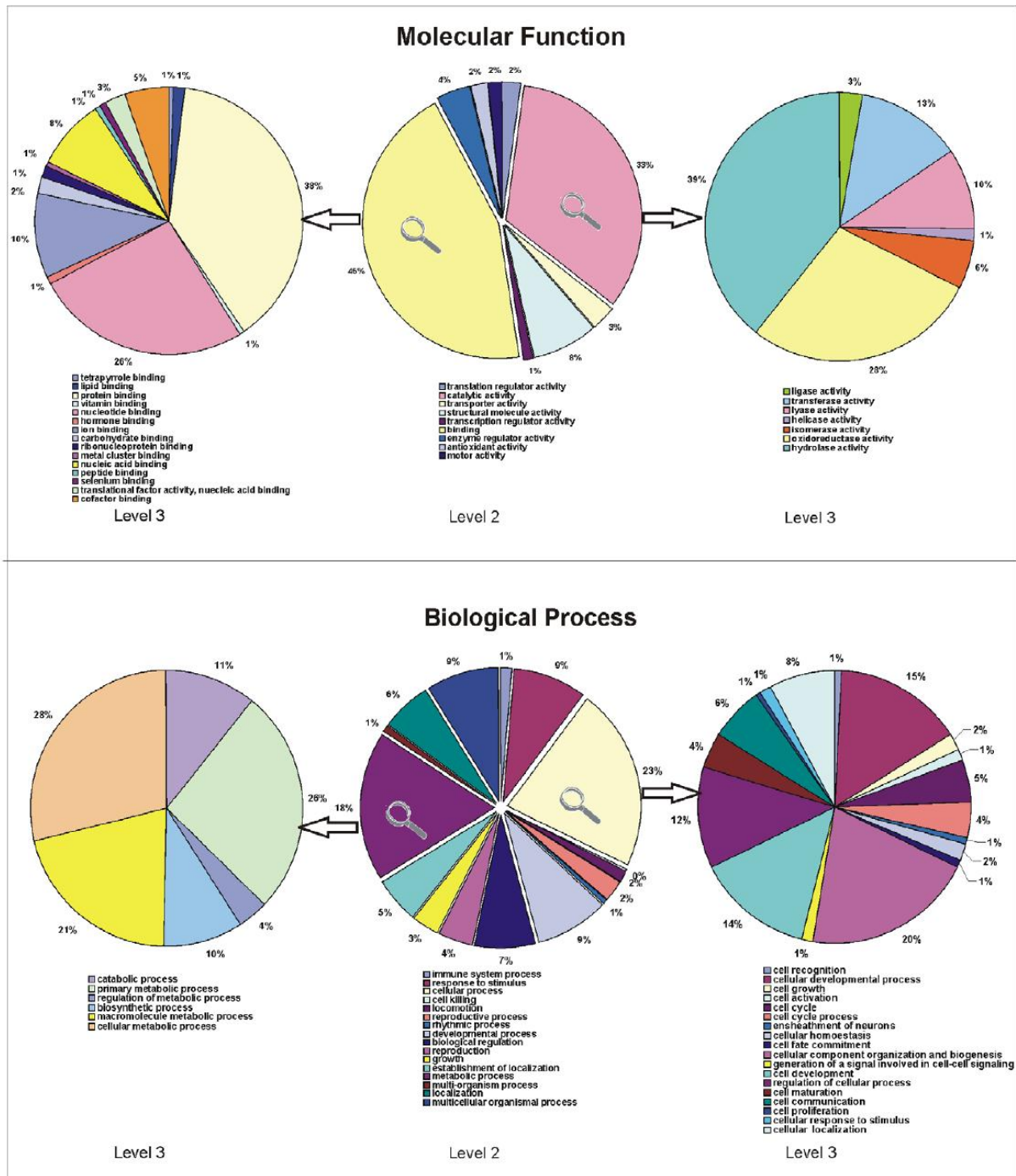
Three different cathepsin proteins could be identified: cathepsin K (spot A84), cathepsin Z (spot E80) and cathepsin L1 (spot F81). Cathepsin L is a ubiquitous cysteine protease in eukaryotes and has been reported as an essential protein for development in *Xenopus laevis* [32], *Caenorhabditis elegans* [33] and *Artemia franciscana* [34].

Several protein spots are associated with ATP generation and consumption and may have important roles in the early development as described for *Artemia*, because many important metabolic processes require ATP [35,36]. ATP synthase (spot B152) regenerates ATP from ADP and Pi [37]. It consists of two parts: a hydrophobic membrane-bound part (CF0) and a soluble

part (CF1) which consists of five different subunits, alpha, beta (spot E89), gamma, delta (spot C139) and epsilon. Arginine kinase (spot B167) is an ATP/guanidine phosphotransferase that provides ATP by catalyzing the conversion of ADP and phosphorylarginine to ATP and arginine [38]. The presence of arginine kinase has been shown in tissues with high energy demand [39].

Interestingly, we could identify the translationally controlled tumor protein (TCTP) (spot F75) on the 2D gel. TCTP is an important component of TOR (target of rapamycin) signalling pathway, which is the major regulator of cell growth in animals and fungi [40].



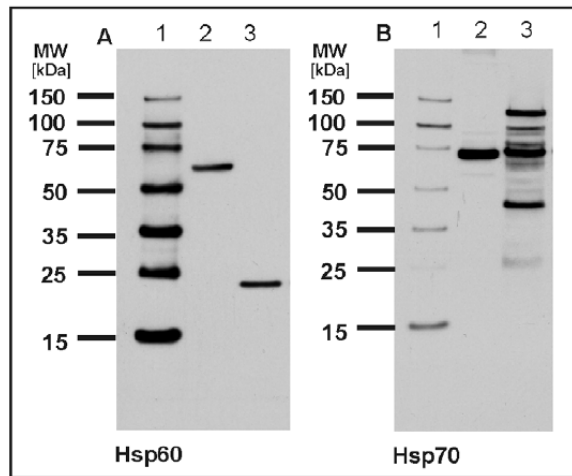


**Figure 7. GO analysis of proteins identified in *M. tardigradum*.** A total of 271 spots representing 144 unique proteins was analysed with the Blast2GO program. The GO categories “molecular function” and “biological processes” are shown as pie charts. A total of 9 different molecular function groups and 16 groups for biological processes are present in our result. The major parts of these categories (level 2) are shown in more detail (level 3) on the left and right side. doi:10.1371/journal.pone.0009502.g007

**Evaluation of Heat Shock Proteins by Western Blot Analysis**

To evaluate the highly conserved heat shock proteins 60 and 70, we performed Western blot analyses with antisera directed against

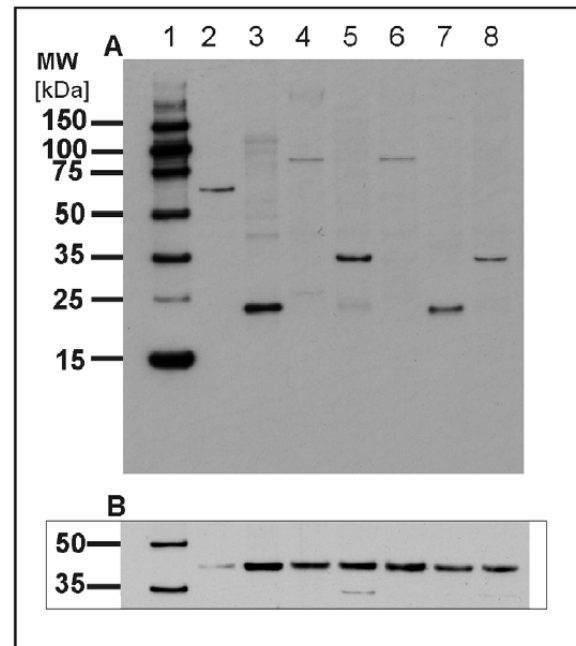
these proteins. Hsp70 was found in several spots on the reference 2D proteome map, e.g. in spot B172, C31, C133 and F27. None of these spots fits well to the calculated molecular weight of approx. 70 kDa, most of them were considerably smaller. In contrast, the



**Figure 8. Detection of hsp60 and hsp70 by Western blotting.** Total protein extract of *M. tardigradum* in the active state was separated on a one-dimensional polyacrylamide gel. Hsp60 (A) and hsp70 (B) could be immunodetected with high sensitivity. Lane 1A and 1B: DualVue Western blotting marker. Lane 2A and 2B: Total protein extract of HeLa cells. Lane 3A and 3B: Total protein extract of tardigrades. Notably, the protein bands in the HeLa control lysate show molecular weights of 60 and 70 kDa as expected. In contrast the detected protein band for hsp60 in *M. tardigradum* is considerably smaller. For hsp70 multiple bands are observed in *M. tardigradum* at higher as well as at lower molecular weights.  
doi:10.1371/journal.pone.0009502.g008

immunoblot shows the strongest band at the expected position which is in agreement with the position of hsp70 in the control lysate of HeLa cells (Figure 8B). However, several additional bands can be observed at higher as well as at lower molecular weights. The lower bands might account for the identified spots on the 2D gel with lower molecular weight. The full-length protein might have escaped the spot picking procedure since only a limited number of detected spots were further processed.

Hsp60 was identified in spot F57 of the 2D map as described above. Since hsp60 was identified by only one peptide hit we confirmed this result by immunostaining using an antibody directed against a peptide in the C-terminal region of the entire protein. Only one band is visible on the Western blot at approx. 24 kDa whereas the protein band in the HeLa control lysate is located at its expected position (Figure 8A). The lower molecular weight is in accordance with the location of hsp60 (spot F57) on the 2D gel. Thus, in *M. tardigradum* hsp60 exists in a significantly shorter form. Whether the observed difference in the molecular weight indicates a different function and role of this protein in *M. tardigradum* needs to be investigated in future experiments. To test whether other tardigrade species show similar results we performed an immunoblot with protein lysates from 5 other species namely *Paramacrobriotus richtersi*, *Paramacrobriotus "richtersi group" 3*, *Macrobriotus tonollii*, *Paramacrobriotus "richtersi group" 2* and *Paramacrobriotus "richtersi group" 1*. Total protein lysate from HeLa cells was loaded as control (Figure 9A, lane 1). Actin served as loading control for all lysates (Figure 9B). Interestingly, some species also exhibit truncated forms of hsp60 on the Western blot whereas others show higher forms. The molecular weights are ranging from approx. 75 kDa for *P. "richtersi group" 2* and *P. "richtersi group" 1* lysates (Figure 9A, lane 4 and 6), 35 kDa for *P. "richtersi group" 3* and *P. richtersi* lysates (Figure 9A, lane 5 and 8) down to 24 kDa for *M. tardigradum* and *M. tonollii* (Figure 9, lane 3 and 7).



**Figure 9. Detection of hsp60 in six different tardigrade species by Western blotting.** Total protein extracts of tardigrades in the active state were separated on a one-dimensional polyacrylamide gel. Hsp60 (A) and actin (B) as loading control were immunodetected with high sensitivity. Lane 1: DualVue Western blotting marker. Lane 2: Total protein extract of HeLa cells. Lane 3: Total protein extract of *M. tardigradum*. Lane 4: Total protein extract of *Paramacrobriotus richtersi*. Lane 5: Total protein extract of *Paramacrobriotus "richtersi group" 3*. Lane 6: Total protein extract of *Macrobriotus tonollii*. Lane 7: Total protein extract of *Paramacrobriotus "richtersi group" 2*. Lane 8: Total protein extract of *Paramacrobriotus "richtersi group" 1*. Interestingly, the detected protein bands were ranging from 100 kDa to less than 24 kDa. Only hsp60 in the HeLa control lysate was detected at its expected position at 60 kDa.  
doi:10.1371/journal.pone.0009502.g009

## Discussion

### Establishing a Comprehensive Proteome Map of *Milnesium tardigradum*

The analysis of the proteome of *M. tardigradum* represents to our knowledge the first detailed study of tardigrades on the protein level. Our experimental strategy aimed to identify as many as possible proteins from tardigrades. Thus, we have not employed any subcellular fractionation steps to obtain specific subproteomes. We have tested various protocols for protein extraction from whole tardigrades. We could show that direct homogenisation of tardigrades in lysis buffer without any previous precipitation steps is most efficient and enables the generation of high quality 2D gels. Since nothing was known about the proteolytic activity in *M. tardigradum* special precautions were taken to avoid any protein degradation or proteolysis throughout the whole workup procedure. Integrity of proteins was carefully inspected by Western blot analysis of the two housekeeping proteins actin and tubulin where the sequence homology was assumed to be high enough to detect the proteins with commercially available antibodies. The development of a robust workup protocol laid the basis for the generation of a protein map from whole tardigrades in the active state. 56 unique proteins could be identified by searching high

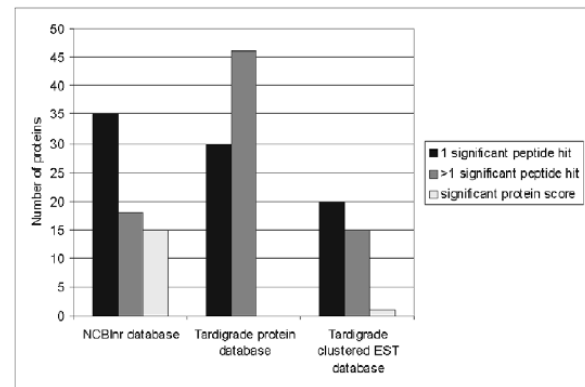
quality MS/MS spectra against the publicly available NCBI database. However, for many proteins we could not find any homologues in the NCBI database and only by using our own newly generated tardigrade protein database it was possible to identify another 73 unique proteins. 15 proteins were present in both databases. In addition 36 unique proteins were found in the clustered tardigrade EST database which could not be annotated by BLAST search. This concerns new specific proteins of *M. tardigradum*.

### Performance of Database Searches

When we started our study of the tardigrade proteome very little was known about tardigrades at the genome and gene expression level. To this day, only 12 proteins are recorded in the NCBI database, which originate from *M. tardigradum*. For all of them only partial sequences ranging from as few as 43 amino acids for beta actin up to 703 amino acids for elongation factor-2 are available. Therefore, in parallel to our proteomic study a *M. tardigradum* EST sequencing project has been initiated. Subsequently, two tardigrade specific databases have been established: a clustered tardigrade EST database and a tardigrade protein database which was extracted from the clustered EST database and thus represents a subdatabase containing all tardigrade-specific proteins with annotated function. However, since cDNA sequencing is still ongoing sequence information remains incomplete. We assume that the tardigrade database currently covers approximately one tenth of the tardigrade specific genes comparing the unique clusters found in tardigrades to all known proteins of *Caenorhabditis elegans* or *Drosophila melanogaster* in Ensembl. This fact is greatly influencing our database searches. For most of the protein spots that were analysed by ESI-MS/MS high quality fragmentation spectra were obtained from MS/MS experiments. However, when we searched these MS/MS data against the tardigrade databases and the publicly available NCBI database, only about 70% of the spots yielded in protein identification whereas the remaining spots gave no significant protein hit. In addition it was impossible to manually extract peptide sequences that were sufficient in length to perform BLAST searches with satisfactory results.

When we examined the protein hits obtained by the three databases in more detail we found that in the NCBI database approximately one half of the proteins were identified by only one significant peptide hit (Figure 10). For about 25% of the proteins more than one significant peptide hit was obtained. For the remaining 25% only the protein score which is the sum of two or more individual peptides scores was above the significance threshold while none of the peptide scores alone reached this value. In contrast, proteins found in the tardigrade protein database were predominantly identified by more than one significant peptide hit whereas a smaller number was represented by only one peptide. In no cases a protein was identified by the sum of non-significant peptide matches. For proteins without annotation the number of proteins identified by only one peptide was only slightly higher than the number of proteins identified by two or more peptides.

These results are not surprising. Since the NCBI database contains very few sequences originating from *M. tardigradum* e.g. elongation factor 1-alpha the identification relies predominantly on high homologies between tardigrade sequences and sequences from other more or less related species of other taxa. The chances for detecting more than one identical peptide is significantly higher when searching MS/MS data against the tardigrade EST and tardigrade protein databases since these databases contain only tardigrade specific sequences.



**Figure 10. Statistical analysis of significant peptides found in the three different databases which were used to search the MS/MS data.** The number of significant peptide hits is compared between the different databases. When searching against the NCBI database most proteins were identified with only one significant peptide hit. In contrast when using the tardigrade protein database most proteins were represented by two or more significant peptides. doi:10.1371/journal.pone.0009502.g010

Overall, one might evoke a potentially high false positive rate especially since proteins are included in the reference map which are either identified by only one significant peptide hit or where two or more non-significant peptide scores are summed up to a significant protein score. On the other hand, proteins like LEA and heat shock protein 60 are identified by only one peptide match. Nevertheless they could be confirmed by Western blot analysis to be present in the tardigrade protein extract. Given the incomplete sequence data available to date many proteins might escape confirmation by orthogonal methods e.g. due to the lack of specific antibodies.

### Proteins Associated with Anhydrobiosis

Among the numerous proteins which were identified in our proteomic study some proteins have already been reported to play an important role in anhydrobiotic organisms. Most importantly, spot F88 was identified as a protein belonging to the LEA (late embryogenesis abundant) family (group 3). This result was already known from Western blot analyses (Schill et al., 2005, poster presentation, ISEPEP, Denmark). At least six different groups of LEA proteins have been described so far. Group 1, 2 and 3 are the three major groups. Whereas group 1 is only found in plants and group 2 predominantly in plants, group 3 is reported in organisms other than plants. Although the precise role of LEA proteins has not yet been fully elucidated, different research groups have reported on their association with tolerance to water stress by desiccation [41,42]. LEA protein of group 3 could be already identified in nematodes *C. elegans*, *Steinernema feltiae* and *Aphelenchus avenae*, and the prokaryotes *Deinococcus radiodurans*, *Bacillus subtilis* and *Haemophilus influenzae* [43,44,45].

### Proteins Exhibiting an Unusual Location on the 2D Map

In general we identified some proteins which show a lower molecular weight than expected. As described above hsp60 is detected as a protein band at 24 kDa by Western blotting and the location of the corresponding spot on the 2D gel shows the same molecular weight. Comparison of different tardigrade species indicates the existence of short as well as long forms of hsp60.

Unique proteins, when analyzed on the 2D gel, often show multiple spots due to posttranslational modifications. Proteins of the vitellogenin family are widely distributed on the 2D gel and show pI as well as molecular weight shifts, which are due to modification through cleavage and to different PTMs like glycosylation and phosphorylation during development of oocytes. Ongoing experiments to detect PTMs using different fluorescence staining methods like ProQ-Emerald for the detection of glycoproteins and ProQ-Diamond for the detection of phosphoproteins indicate that these modifications indeed occur in tardigrades (data not shown).

### Prediction of Functional Domains in Proteins with Yet Unknown Functions

36 proteins which could not be identified by BLAST searches were further examined looking for matching functional protein domains with DomainSweep. The function of the following two spots could be revealed with high confidence (Table 3): spot F63 seems to belong to the “tumor protein D52” interpro family (IPR007327). The hD52 gene was originally identified through its elevated expression level in human breast carcinoma, but cloning of D52 homologues from other species has indicated that D52 may play roles in calcium-mediated signal transduction and cell proliferation. Regarding the taxonomic neighbours of the tardigrades, one member in *C. elegans* and 10 members in *Drosophila melanogaster* are reported by Interpro for this family. Spot C95 seems to belong to the family “glucose/ribitol dehydrogenase” (IPR002347). 80 members both in *C. elegans* and in *Drosophila melanogaster* are reported for this family. 28 putative hits were found associated with other spots. These protein hits are putative candidates and therefore less reliable. A comprehensive protein database of *M. tardigradum* as the result of our ongoing cDNA sequencing will help us to evaluate these candidates.

### Conclusion

In this study we present for the first time a comprehensive proteome map of *M. tardigradum*. A full description of proteins present in the active state provides a valuable basis for future studies. Most importantly, the protein reference map allows us to undertake quantitative proteomics analysis to detecting proteins with different expression levels in the active versus the anhydrobiotic state. In particular, our workflow is fully compatible with the application of 2D difference gel electrophoresis (2D DIGE), which is one technique allowing sensitive analysis of differences in the protein expression levels. This differential analysis on the protein level will help us to understand survival mechanisms in anhydrobiotic organisms and eventually to develop new methods for preservation of biological materials.

## Materials and Methods

### Tardigrade Culture and Sampling

Tardigrades of the species *M. tardigradum* Doyère 1840 were maintained in a laboratory culture. The culture was grown on agarose plates (3%) (peqGOLD Universal Agarose, peqLAB, Erlangen Germany) covered with Volvic™ water (Danone Waters, Wiesbaden, Germany) at 20°C. The juveniles were fed on green algae *Chlorogonium elongatum*, the adults with bdelloid rotifers *Philodina citrina*. The specimens for the experiments were all of middle-age, thus effects of age can be excluded. Tardigrades were starved over 3 days and washed several times with Volvic™ water to avoid contamination with food-organisms. Subsequently the animals were transferred to microtiter tubes (200 individuals per tube) and surrounding water was reduced to approx. 1–2 µl.

An active state (I) according to Schill et al. [18] was investigated in this work. All samples were shock frozen in liquid nitrogen and stored at –80°C. 200 individuals are defined as one aliquot. Other tardigrade species (*Paramacrobrotus richtersi*, *Paramacrobrotus “richtersi group”* 3, *Macrobrotus tonollii*, *Paramacrobrotus “richtersi group”* 2 and *Paramacrobrotus “richtersi group”* 1) used for immunodetection of hsp60 were prepared in the same way.

### Sample Preparation for Gel Electrophoresis

To optimize the sample preparation different precipitation methods have been tested. Chloroform/methanol and TCA/acetone precipitations were performed as described by Wessel, Flügge [46] and Görg [47], respectively. We used also the commercially available precipitation kit (clean-up kit from GE Healthcare). Comparing the result of different precipitation protocols on a 1D gel we decided to homogenise the tardigrades directly in ice cold lysis buffer and avoid any precipitation steps. The animals (200 individuals) were homogenised directly in 60 µl lysis buffer (containing 8 M urea, 4% CHAPS, 30 mM Tris, pH 8.5) by ultrasonication (SONOPULS, HD3100, Bandelin Electronic) with 45% amplitude intensity and 1–0.5 sec intervals. The lysis buffer contained a Protease Inhibitor Mix (GE Healthcare) to inhibit serine, cysteine and calpain proteases. After homogenisation the samples were stored at –80°C. For gel electrophoresis insoluble particles were removed by centrifugation for 2 min at 14,000 g and the supernatant was quantified using BCA mini-assay.

### One Dimensional Gel Electrophoresis and Western Blotting

To compare the efficiency of different sample preparation methods we separated approx. 10 µg total protein extract on a 1D gel. The gel was stained with protein staining solution (PageBlue from Fermentas). For Western blotting a total protein extract of tardigrades (15–20 µg) was separated on a NuPAGE™ 4–12% Bis-Tris mini gel (Invitrogen) using MES running buffer. 200 V were applied until the bromophenol blue front had reached the bottom of the gel (approx. 40 min). Separated proteins were electro transferred onto PVDF membrane for 1.5 h at maximum 50 mA (0.8/cm<sup>2</sup>) in a semi-dry transfer unit (Hoefer™ TE 77) using following transfer solution: 24 mM Tris, 192 mM glycine and 10% methanol. The PVDF membrane was incubated in a blocking buffer containing 5% non-fat milk, 0.1% Tween20 in PBS. As primary antibodies we used anti actin pan Ab-5 (dianova), anti hsp 60 Ab (D307) (Cell signaling), anti hsp70 Ab (BD Biosciences Pharmingen) and anti α-Tubulin Ab (Sigma).

For molecular weight determination of the target proteins on film we used ECL DualVue marker (GE-Healthcare). Immunoreaction was detected using the ECL Western Blotting Detection kit from GE Healthcare. Images were acquired using an Image Scanner Model UTA-1100 (Amersham Biosciences).

### Two Dimensional Gel Electrophoresis

For 2D gel preparation we added 60 µl 2x sample buffer (7 M urea, 2 M thiourea, 2% CHAPS, 2% DTT, 2% IPG-buffer 3–11 NL) to each aliquot and incubated by shaking for 30 min at 25°C. To avoid streaking on the gels we used 330 µl destreaking buffer (GE Healthcare) instead of rehydration buffer, to which we added 2% IPG-buffer (pI 3–11). Samples were incubated by shaking for 30 min at 25°C. We loaded 100 µg protein on analytical gels and 330 µg on preparative gel.

**Strip loading.** Loading of proteins was performed during strip rehydration with the recommended volume (450 µl for 24 cm strips) over night.

**IEF conditions.** First dimension isoelectric focusing (IEF) was performed, using 24 cm long IPG strips with non-linear gradients from pH 3–11 and an Ettan IPGphor instrument and proceeded for 46.4 kVh with the following running protocol: 3 h at 300 V, 6 h at 500 V, 8-h gradient up to 1000 V, 3-h gradient up to 8000 V and 3 h at 8000 V. Strips were either immediately used for the second dimension or stored at  $-80^{\circ}\text{C}$ .

**Second dimension.** Strips were equilibrated in 6 M urea, 2% SDS, 30% glycerol, 0.375 M Tris-HCl pH 8.8, 0.002% bromophenol blue and 10 mg/ml DTT for 15 min, followed by a second equilibration step with the same buffer containing 25 mg/ml iodoacetamide instead of DTT, also for 15 min.

Strips were loaded on 12% SDS-gels with an overlay of agarose solution (0.5 mg/100 ml electrophoresis buffer). The second dimension was performed using an Ettan Dalttwelve electrophoresis system (GE Healthcare). Separation was carried out at 1.5 watt/1.5 mm thick gel until the bromophenol blue reached the bottom of the gel (approx. 18 h).

**Silver staining of proteins and image analysis.** Proteins on analytical gels were visualized by destructive silver staining according to Blum [48]. Additionally, we performed a silver stain compatible with mass spectrometric analysis described by Sinha [49] for preparative gels. Images were acquired using an Image Scanner Model UTA-1100 (Amersham Biosciences).

## Protein Identification

**In-gel digestion.** Protein spots were excised semi-manually with a spot picker (GelPal, Genetix) following non-destructive silver staining and stored at  $-80^{\circ}\text{C}$  after removing water. Gel pieces were reduced, alkylated and in-gel digested with trypsin. Briefly, after incubation with 150  $\mu\text{l}$  water at  $42^{\circ}\text{C}$  for 8 min, water was removed (washing step) and gel pieces were shrunk by dehydration with 150  $\mu\text{l}$  40 mM  $\text{NH}_4\text{HCO}_3$ /ethanol 50:50 (v/v) at  $42^{\circ}\text{C}$  for 5 min in a thermo mixer (600 rpm). The solution was removed and the proteins were reduced with 50  $\mu\text{l}$  10 mM dithiothreitol in 40 mM  $\text{NH}_4\text{HCO}_3$  for 1 h at  $56^{\circ}\text{C}$ . The solution was removed and gel pieces were incubated with 150  $\mu\text{l}$  40 mM  $\text{NH}_4\text{HCO}_3$  for 5 min at  $42^{\circ}\text{C}$ . After removing the solution gel pieces were alkylated with 100  $\mu\text{l}$  55 mM iodoacetamide in 40 mM  $\text{NH}_4\text{HCO}_3$  for 30 min at  $25^{\circ}\text{C}$  in the dark, followed by three alternating washing steps each with 150  $\mu\text{l}$  of 40 mM  $\text{NH}_4\text{HCO}_3$  and ethanol for 5 min at  $37^{\circ}\text{C}$ . Gel pieces were then dehydrated with 100  $\mu\text{l}$  neat acetonitrile for 1 min at room temperature, dried for 15 min and subsequently rehydrated with porcine trypsin (sequencing grade, Promega, Mannheim, Germany) with the minimal volume sufficient to cover the gel pieces after rehydration (100 ng trypsin in 40 mM  $\text{NH}_4\text{HCO}_3$ ). Samples were incubated over night at  $37^{\circ}\text{C}$ .

**Extraction.** After digestion over night the supernatant was collected in PCR-tubes while gel pieces were subjected to four further extraction steps. Gel pieces were sonicated for 5 min in acetonitrile/0.1% TFA 50:50 (v/v). After centrifugation the supernatant was collected and gel pieces were sonicated for 5 min in acetonitrile. After collecting the supernatant gel pieces were sonicated for 5 min in 0.1% TFA followed by an extraction step again with acetonitrile. The combined solutions were dried in a speed-vac at  $37^{\circ}\text{C}$  for 2 h. Peptides were redissolved in 6  $\mu\text{l}$  0.1% TFA by sonication for 5 min and applied for ESI-MS/MS analysis.

**ESI-MS/MS analysis and database search.** NanoLC-ESI-MS/MS was performed on a Qtof Ultima mass spectrometer (Waters) coupled on-line to a nanoLC system (CapLC, Waters). For each measurement 5  $\mu\text{l}$  of the digested sample was injected. Peptides were trapped on a Trapping guard C18-AQ

10 mm $\times$ 0.3 mm, particle size 5  $\mu\text{m}$  (Dr. Maisch). The liquid chromatography separation was performed at a flow rate of 200 nl/min on a Reprosil C18-AQ column, 150 mm $\times$ 75  $\mu\text{m}$ , particle size 3  $\mu\text{m}$  (Dr. Maisch GmbH). The following linear gradient was applied: 5% B for 5 min, from 5 to 15% B in 5 min, from 15 to 40% B in 25 min, from 40 to 60% B in 15 min and finally 60 to 95% B in 5 min. Solvent A contains 94.9% water, 5% acetonitrile, 0.1% formic acid, solvent B contains 95% acetonitrile, 4.9% water and 0.1%  $\mu\text{l}$  formic acid. The LC-ESI-MS/MS device was adjusted with a PicoTip Emitter (New Objective, Woburn, MA) fitted on a Z-spray nanoESI interface (Waters). Spectra were collected in the positive ion mode. The capillary voltage was set to 2400 V and the cone voltage was set to 80 V. Data acquisition was controlled by MassLynx<sup>TM</sup> 4.0 software (Waters). Low-energy collision-induced dissociation (CID) was performed using argon as a collision gas (pressure in the collision cell was set to  $5\times 10^{-5}$  mbar), and the collision energy was in the range of 25–40 eV and optimized for all precursor ions dependent on their charge state and molecular weight. Mass Lynx raw data files were processed with Protein Lynx Global Server 2.2 software (Waters). Deisotoping was performed using the MaxEnt3 algorithm.

The obtained MS/MS spectra were searched against the publicly available NCBI database using the MASCOT algorithm version 2.0 (Matrix Science, London, UK). The mass tolerance was set to 0.1 Da for fragment ions and 200 ppm for precursor ions. No fragment ions score cutoff was applied. The following search parameters were selected: variable modification due to methionine oxidation, fixed cysteine modification with the carbamidomethyl-side chain, one missed cleavage site in the case of incomplete trypsin hydrolysis. The following settings were applied: minimum protein score  $>53$ , minimum number of peptides  $\geq 1$ . Furthermore, protein hits were taken as identified if a minimum of one peptide had an individual ion score exceeding the MASCOT identity threshold. Under the applied search parameters a sum MASCOT score of  $>53$  refers to a match probability of  $p<0.05$ , where  $p$  is the probability that the observed match is a random event. Redundancy of proteins that appeared in the database under different names and accession numbers was eliminated. Additionally we searched against the *M. tardigradum* EST and protein database (see below) to identify sequences not present in the NCBI databases. The following settings were applied: minimum protein score  $>14$  for the EST and  $>27$  for the clustered EST database ( $p<0.05$ ). Other parameters were as described for the NCBI searches.

## Generation of the Tardigrade EST Database

cDNA libraries from mRNA from tardigrades in different states (active, inactive, transition states) were prepared and sequenced (Mali et al, submitted data). The obtained EST sequences were cleaned from vector sequences using Seqclean against UniVec-database from NCBI (version 12, September 2008, Kitts et al., unpublished). Repeats within the cleaned ESTs were masked using the online service RepeatMasker (version 3.2.6, RM-20080801, Smit et al., unpublished data) followed by a second Seqclean run to eliminate low quality and short sequences. The assembly was performed using cap3 [50] with clipping enabled and resulted in 3318 Unigenes (2500 singlets, 818 contigs). Identification of ribosomal sequences was done using a BlastN-search [51] against the Silva-DB (only eukaryotic sequences, Silva95, [52]) and an E-value cutoff of  $1e-3$  and resulted in 46 sequences which showed high similarity to ribosomal sequences. Unigenes coding for known proteins were identified using a BlastX search against Uniprot/Swissprot (version 14.1, September 2008), Uniprot/TrEMBL

(version 56.1, September 2008, The UniProt Consortium, 2008) and NRDB (version 12, September 2008,) with an E-value cutoff of  $1e^{-3}$  and a hmmer-search against PFAM database (release 22, [53]) with an E-value cutoff of 0.1. Translation of Unigen sequences which gave a BlastX or PFAM hit (1539/1889 sequences) into the corresponding frame and a six-frame translation was performed using Virtual Ribosome (version 1.1 Feb-Mar, 2006, [54]). For six frame translation the read through mode of Virtual Ribosome was used. Afterwards stop codons were substituted by an undefined amino acid (X). All new sequences have been deposited in GenBank. The accession numbers are indicated in the Tables 2, 3 and S1 in the column “Tardigrade specific Accession no.”.

### Classification of Proteins

For functional analysis of identified proteins we used Blast2GO software, which consists of three main steps: blast to find homologous sequences, mapping to collect GO-terms associated to blast hits and annotation to assign functional terms to query sequences from the pool of GO terms collected in the mapping step [55]. Function assignment is based on GO database. Sequence data of identified proteins were uploaded as a multiple FASTA file to the Blast2GO software. We performed the blast step against public database NCBI through blastp. Other parameters were kept at default values: e-value threshold of  $1e^{-3}$  and a recovery of 20 hits per sequence. Furthermore, minimal alignment length (hsp filter) was set to 33 to avoid hits with matching region smaller than 100 nucleotides. Qblast-NCBI was set as Blast mode. Furthermore, we have chosen an annotation configuration with an e-value-Hit-filter of  $1.0E^{-6}$ , Annotation CutOff of 55 and GO weight of 5. For visualizing the functional information (GO categories: Molecular Function and Biological process) we used the analysis tool of the Blast2GO software.

### Protein Domain Analysis of Proteins without Annotation

Six frame translations of the Unigenes were run through the DomainSweep pipeline [56] and the significant and putative hits were collected. For each of the protein/domain databases used,

different thresholds and rules were established [56]. Domain hits are listed as ‘significant’

- i. if two or more hits belong to the same INTERPRO [57] family. The task compares all true positive hits of the different protein family databases grouping together those hits, which are members of the same INTERPRO family/domain.
- ii. if the motif shows the same order as described in PRINTS [58] or BLOCKS [59]. Both databases characterize a protein family with a group of highly conserved motifs/segments in a well-defined order. The task compares the order of the identified true positive hits with the order described in the corresponding PRINTS or BLOCKS entry. Only hits in correct order are accepted.

All other hits above the trusted thresholds are listed as ‘putative’. By comparing the peptides which were identified by mass spectrometry with the six translations, the correct frame and the associated domain information was listed.

### Supporting Information

**Table S1** Blast2GO analysis of identified proteins. Spot number, protein annotation, accession number and GO information in all three categories molecular function, biological process and cellular component are listed.

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### Author Contributions

Conceived and designed the experiments: ES. Performed the experiments: ES. Analyzed the data: ES AHW. Contributed reagents/materials/analysis tools: AHW UW BM MF FF TD SH ROS. Wrote the paper: ES MS. Supervised the project: MS.

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## 6.2

### **Investigating heat shock proteins of tardigrades in active *versus* anhydrobiotic state using shotgun proteomics**

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Running Head: Proteomic analysis of heat shock proteins from *Milnesium tardigradum*.

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## Abstract

The eutardigrade *Milnesium tardigradum* can undergo cryptobiosis and can survive extreme environmental conditions. However, the survival mechanisms of tardigrades are still poorly understood. Heat shock proteins as an important subgroup of chaperones which protect proteins from irreversible aggregation and degradation might play an important role in anhydrobiosis. In this report we therefore investigated heat shock proteins in tardigrades in the active *versus* the anhydrobiotic state employing proteomics techniques. Protein lysates from tardigrades in both states were separated by one dimensional gel electrophoresis, in-gel digested with trypsin and tryptic peptides were analyzed by high sensitive nanoLC ESI-MS/MS on a LTQ-Orbitrap mass spectrometer. The present study indicates the presence of heat shock proteins of the major chaperone families Hsp40, Hsp60, Hsp70, Hsp90, small Hsps and furthermore nucleotide exchange factors and co-chaperones in *Milnesium tardigradum*. A comparative analysis of the identified heat shock proteins in both states was performed by calculating the exponentially modified protein abundance index (emPAI).

**Key words:** Tardigrades, heat shock proteins, Hsp60 (chaperonin), Hsp40 (DnaJ), Hsp70 (DnaK), Hsp90, small heat shock proteins, nucleotide exchange factors, co-chaperones, high throughput proteomics

## Introduction

One key step for cell survival under stress condition is maintaining proteins in their functional confirmation and preventing protein aggregation. The effector proteins in protection mechanisms developed by exposure to environmental challenges such as heat stress and desiccation are referred to heat shock proteins (Laksanalamai & Robb, 2004; Morimoto, 1994). Heat shock proteins (Hsps) are ubiquitous, highly conserved proteins found in eukaryotes and prokaryotes. Hsps can be divided into diverse families based on their molecular weights. Each class includes the members of a single protein family or related protein families. Many heat shock proteins function as molecular chaperones and are involved in catalyzing the refolding of denatured proteins or folding of newly synthesized proteins and preventing protein aggregation (Laksanalamai & Robb, 2004). Whereas Hsp activity is required under normal cellular conditions, expression is especially critical in stressed cells for survival (Connolly & Hall, 2008; Young *et al.*, 2001). Not only the function of specific chaperone family members is of major importance, but also the chaperone networks. Well known examples are the cooperation between the Hsp90 and Hsp70 machineries during the activation of steroid hormone receptors and the cooperation between the Hsp70 and GroE (nucleotide exchange factor) in protein folding (Walter & Buchner, 2002). In this study we have focused on the heat shock proteins of *Milnesium tardigradum*. Tardigrades are well known because of their capability to undergo

cryptobiosis and survive physical extremes including high and subzero temperatures (Hengherr *et al.*, 2009; Ramlov & Westh, 1992; Ramløv & Westh, 2001), high pressure (Ramløv & Westh, 2001; Seki & Toyoshima, 1998), and extreme levels of ionizing radiation (Horikawa *et al.*, 2006; Jönsson & Schill, 2007). Interestingly, tardigrades are able to survive space vacuum (imposing extreme desiccation) and some specimens have even recovered after combined exposure to space vacuum and solar radiation (Jönsson *et al.*, 2008). Although tardigrades are known for more than 300 years, the molecular mechanisms of this protection against extreme conditions are still poorly understood. Partial gene sequences of three heat shock protein (Hsp70 family) and the housekeeping gene beta-actin have been described (Schill *et al.*, 2004) and the relation of Hsp70 expression to desiccation tolerance could be shown by real time PCR (Schill *et al.*, 2004) and by *de novo* protein synthesis (Ramløv & Westh, 2001). A recently published transcriptome analysis of tardigrades describes the presence of different heat shock proteins like Hsp70, DnaJ proteins in *M. tardigradum* (Forster *et al.*, 2009). However, no in-depth proteomic analysis of Hsps of tardigrades has been published so far. With our investigation we intended to fill this gap by performing shotgun proteomics on tardigrades in the active and anhydrobiotic state using 1D SDS-PAGE and nanoLC-ESI-MS/MS. Our study not only presents the identification of members of major chaperone families (Hsp40, Hsp60, Hsp70, Hsp90 and small Hsps) but also the presence of nucleotide exchange factors and co-chaperones (GrpE, DnaJ). Calculating the exponentially modified protein abundance index (emPAI) of each protein allowed us a comparative analysis of the expression of heat shock proteins in the active *versus* the anhydrobiotic state.

## Materials and methods

### Tardigrade culture and sampling

Tardigrades of the species *Milnesium tardigradum* Doyère 1840 were maintained in a laboratory culture as described elsewhere (Schill *et al.*, 2004). Briefly, the culture was grown on agarose plates (3%) (peqGOLD Universal Agarose, peqLAB, Erlangen Germany) covered with Volvic™ water (Danone Waters, Wiesbaden, Germany) at 20°C. The juveniles were fed on green algae *Chlorogonium elongatum*, the adults with bdelloid rotifers *Philodina citrina*. The specimens for the experiments were all of middle-age, thus effects of age can be excluded. Tardigrades were starved over 3 days before harvesting and washed several times with Volvic™ water to avoid contamination with food organisms. Subsequently the animals were transferred to microliter tubes (200 individuals per tube) and surrounding water was reduced to approx. 1-2 µl. Active (I) and anhydrobiotic states (III) according to Schill *et al.* (Schill *et al.*, 2004) were investigated in this work. For the induction of the anhydrobiotic state (III), animals were desiccated in open microliter tubes (Biosphere SafeSeal Micro Tubes, Sarstedt, Nümbrecht, Germany) exposed to 85% relative humidity (RH) in a chamber containing a saturated solution of KCl (Roth, Karlsruhe,

Germany) at 21°C for 24 h, subsequently transferred to a chamber containing a saturated MgCl<sub>2</sub> solution (Roth, Karlsruhe, Germany), where they were exposed to 33% RH for at least 48 h. All samples were frozen in liquid nitrogen and stored at -80°C.

#### Sample preparation and one dimensional gel electrophoresis

The animals (200 individuals) were homogenized in 60 µl lysis buffer containing 8M urea, 4% CHAPS, 30 mM Tris and Protease Inhibitor Mix (GE Healthcare, München, Germany), pH 8,5 by ultrasonication (SONOPULS, HD3100, Bandelin Electronic) with 45% amplitude intensity and 1-0.5 sec intervals. After homogenization the samples were shock frozen and stored at -80°C. For gel electrophoresis insoluble particles were removed by centrifugation for 2 min at 14,000g and the supernatant was quantified using BCA mini-assay. One dimensional gel electrophoresis was performed using precast 4–12% Bis-Tris mini gels (Invitrogen, Karlsruhe, Germany) in MES buffer system. Gels were loaded with 40 µg protein per lane and stained using protein staining solution from Fermentas (St. Leon-Rot, Germany). The entire lane was cut into 27 equal slices except slice 26 and 27, which were twice as large, and used for in-gel digestion with trypsin.

#### Protein identification

Tryptic digestion and extraction were performed as recently described (Schokraie *et al.*, 2010). After extraction the solutions were dried in a speed-vac at 37°C for 2 h. Peptides were redissolved in 5 µl 0.1% TFA by sonication for 15 min and were applied for separation using a nano Acquity UPLC (Waters GmbH, Eschborn, Germany). Peptides were trapped on a nano Acquity C18 column, 180 mm x 20 mm, particle size 5 µm (Waters GmbH, Eschborn, Germany). The liquid chromatography separation was performed at a flow rate of 400 nl/min on a BEH 130 C18 column, 100 mm x 100 µm, particle size 1.7 µm (Waters GmbH, Eschborn, Germany). The following linear gradient was applied: from 0 to 4% B in 1 min, from 4 to 30% B in 80 min, from 30 to 45% B in 10 min, from 45 to 90% B in 10 min, 10 min at 90% B and from 90 to 0% B in 0.1 min. Solvent A contains 98.9% water, 1% acetonitrile, 0.1 % formic acid, solvent B contains 99.9% acetonitrile and 0.1% µl formic acid. The nanoUPLC system was coupled online to a LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Data were acquired by scan cycles of one FTMS scan with a resolution of 60000 and a range from 370 to 2000 m/z in parallel with six MS/MS scans in the ion trap of the most abundant precursor ions.

The mgf-files were used for database searches with the MASCOT search engine (Matrix Science, London, UK; version 2.2) against the NCBI nr database (October 13, 2009, all entries) and a newly developed tardigrade database containing contigs from Sanger and 454 sequencing (unpublished data). The peptide mass tolerance for database searches was set to 5 ppm and fragment mass tolerance to 0,6 Da. Carbamidomethylation of C was set as fixed modification. Variable modifications included oxidation of M and deamidation of NQ. One missed cleavage site in the case of incomplete trypsin hydrolysis was allowed. Furthermore, proteins were considered as identified if a minimum of one peptide had an individual ion score exceeding

the MASCOT identity threshold (NCBI nr score >52, tardigrade database score >24). Identification under the applied search parameters refers to a match probability of  $p < 0.005$  and a false discovery rate of <4% for NCBI nr database search and a match probability of  $p < 0.01$  and a false discovery rate of <2% for tardigrade database search. The abundance of proteins was estimated by comparing the exponentially modified Protein Abundance Index (emPAI) (Ishihama *et al.*, 2005), which was automatically calculated by the MASCOT search engine.

### **Preparation of tardigrade protein database**

#### *Assembly of the 454 sequences together with the assembled Sanger sequences*

1 million reads from the 454 sequencing received by GATC (<http://www.gatc-biotech.com/de/index.html>) together with 7723 already assembled Sanger sequences were assembled using Mira3 ([http://chevreux.org/projects\\_mira.html](http://chevreux.org/projects_mira.html)), with the job parameters de novo, accurate, est, 454, sanger. The assembly yielded 20158 contigs with a length larger than 300 bases and an average coverage of more than 3 (each position in the contig is on average determined by 4 or more reads or sanger sequences). The average length of sequences is 189 amino acids (567 bases).

#### *Prediction of the proteins from the EST sequences*

FrameDP peptide prediction (Gouzy *et al.*, 2009) (version 1.0.3; standard parameters) was performed locally on a 2.4 Ghz quad-core desktop computer with 4 Gb RAM running GNU/Linux (Ubuntu 8.10). The learning set was split using the GC3%-method (i.e. GC content of the third codon position) and FrameDP was trained on *M. tardigradum* coding style against *Drosophila melanogaster* protein data as a reference database (Flybase:dmel-all-translation-r5.21.fasta, [ftp://ftp.flybase.net/genomes/Drosophila\\_melanogaster](ftp://ftp.flybase.net/genomes/Drosophila_melanogaster)). The annotation of the predicted proteins was performed using BlastX search (Altschul *et al.*, 1997) against Uniprot/Swissprot (version 57.7, September 2009), Uniprot/TrEMBL (version 40.7, September 2009, The UniProt Consortium, 2008) and NRDB (version 1. September 2009) with an E-value cut-off of  $1e-3$  and a hmmer2-search against PFAM database (release 23, (Finn *et al.*, 2008) with an E-value cut-off of  $1e-3$ .

### **Multiple alignments and secondary structure prediction**

Multiple alignment for comparing different protein sequences was performed using the ClustalW program (Thompson *et al.*, 1994) with the default parameters. The identity and similarity values were calculated by pair-wise comparison with the Gap program (Wisconsin Package, Accelrys Software Inc.) with the default parameter setting. To obtain the helical domains of proteins from J family we used GOR IV secondary structure prediction method (Garnier *et al.*, 1996) from ExPASy tools (<http://www.expasy.ch/tools/>).

## Result and discussion

### Identification of heat shock proteins

Proteomic analysis of whole protein extracts of tardigrades in the active and anhydrobiotic state included protein separation by 1D gel electrophoresis (figure 1), followed by slicing of the gel in 27 individual slices, in-gel tryptic digestion of proteins in the individual gel slices and slice-by-slice analysis by LC-ESI-MS/MS of tryptic peptides. Identification of proteins by subsequent database searches yielded different heat shock proteins. We found specific peptides for heat shock proteins from different species by searching MS/MS data against the NCBI nr database. The identified protein hits with detailed information including slice number (A indicates active state and T anhydrobiotic (tun) state), accession number, protein annotation, total protein score, number of unique peptides, sequence coverage, protein mass and pI are listed in table 1. Furthermore the emPAI (Exponentially Modified Protein Abundance Index) and sum of emPAI in case we found the same protein hit more than once is included in table 1. Additionally, corresponding peptide sequences with peptide scores are included for each identified protein in the Supplementary Table 1. The comparison of emPAI of different proteins in the sample offers approximate, label-free, relative quantification of proteins in a mixture based on protein coverage by the peptide matches in a database search result (Ishihama *et al.*, 2005). The protein information listed in table 1 belongs to the protein hit with the highest sequence coverage and the number of the corresponding gel slice is given in bold. In case we found the same protein in other gel slices the numbers of these slices are also included. Since to this day only 53 protein entries originating from *M. tardigradum* are recorded in the publicly available NCBI nr database, the identification of proteins is based predominantly on high homologies between tardigrade sequences and sequences from other more or less related species of other taxa. Thus only conserved proteins can be identified with high sequence coverage. Since heat shock proteins (Hsps) are known to be highly conserved, we found many protein hits among different species mainly for Hsp 60, 70 and 90 families. Especially, for the 70 kDa heat shock protein (Hsp70) chaperones, which are conserved from bacteria to mammals (Bukau & Horwich, 1998) the database search delivered queries matches from more than 45 different species (table 1, 10-61). Eukaryotes as well as prokaryotes contain multiple Hsp70 isoforms (Sharma & Masison, 2009). Humans encode at least eight different Hsp70 homologues, two of which are organelle specific and the remaining six reside in the cytosol and nucleus (Sharma & Masison, 2009). The constitutively expressed cytosolic form is Hcs70 (heat shock cognate). Similarly, yeast contains organelle specific and cytosolic Hsp70s. Yeast cytosolic Hsps70s are subdivided into two classes Ssa and Ssb which share approximately 60% identity, although they are functionally different (Craig *et al.*, 1995). Likewise, many prokaryotic organisms contain multiple Hsp70 isoforms; the main Hsp70 is known as DnaK protein.

For the analysis of the proteome of tardigrades the availability of a protein database is a prerequisite. Therefore in parallel to our proteomic studies a cDNA sequencing project was initiated, which is still in progress. EST sequence analysis of tardigrades, assembling the sequences to contigs, translating the sequences to proteins and annotating them by homology search (blast search) allowed us to establish the basis for identification and analysis of proteins of tardigrades. By searching MS/MS data against the tardigrade database, we could identify proteins from heat shock protein families 40, 60, 70, 90 and 20 (small heat shock) with high sequence coverage up to 75%. Detailed information of identified proteins in the tardigrade database is listed in table 2. Additionally, corresponding peptide sequences with peptide scores and protein sequence of identified heat shock proteins are included for each identified protein in the Supplementary Table 2.

For the active as well as the anhydrobiotic state approximately 1000 proteins could be identified by searching MS/MS data against the NCBI nr database. Identified proteins are almost conserved proteins like actin and members of heat shock protein families. Since we selected all entries as search parameter for taxonomy we received many hits for one protein from different species, which means that the 1000 identified proteins are not unique proteins. In contrast by searching MS/MS data against the tardigrade database approximately 2300 unique proteins could be identified for both states. Regarding the heat shock proteins we could identify protein members of heat shock protein 40 family and small heat shock proteins exclusively by searching MS/MS data against the tardigrade protein database.

### Heat shock protein 70 family

For the heat shock protein 70 family we found four contigs (protein no. 17, 18, 21, 22, table 2), which could be annotated as Hsp71 (*Danio rerio*) by blast search. Figure 2A shows the multiple alignment of these four contigs together with the protein sequence of Hsp71 from zebrafish (*Danio rerio*). Identical sequences are highlighted. The high similarity of these contigs between each other suggests the presence of similar but not identical Hsp70 isoforms in *M. tardigradum*. Contig3 (protein no. 21, table 2) and contig4 (protein no. 17, table 2) share the highest similarity compared to other contigs (figure 2B, similarity 80.16%, identity 71.97%). For further analysis we selected the Hsp70 protein hit (protein no. 18 in table 2) with highest sequence coverage (62.1% in active state, 58.1% in anhydrobiotic state) to align the peptide sequences of some identified proteins found by searching MS/MS data against the NCBI nr database. The sequence of Hsp70 of *M. tardigradum* is shown in figure 3A. Peptides identified in the tardigrade database are indicated in bold and are also underlined in red. The peptide sequences which were found in the NCBI nr database are highlighted with lines of different colours for each species. The highly conserved domains are easily recognized by the highest number of repeatedly identified peptides from different species. Since bacterial and mammalian Hsp70s share 50% identity and

have essentially superimposable structures and similar enzymatic functions, Hsp70 chaperones across species are believed to work through similar mechanisms. Hsp70s consist of three structural domains: a 44kDa amino-terminal nucleotide binding domain (NBD, also called ATPase domain) followed by an 18kD substrate binding domain (SBD) and a 10kD C-terminal domain (CTD), which is the least conserved of the three domains and is therefore referred to as the variable domain (Ungewickell *et al.*, 1997). NBD domain (aa 1-383), substrate binding domain (aa 384-543) and C-terminal domain (aa 544-650) are shown in figure 3B for bovine Hsc70 (Ungewickell *et al.*, 1997). Considering the peptide sequences, which were repeatedly identified in the NCBI nr database among different species, we can conclude the presence of at least 5 conserved signatures in the NBD domain of *M. tardigradum* (figure 3A). Furthermore we found Hsp70 isoform 1 and Hsp70 isoform 2, which were described in earlier studies of *M. tardigradum* (Schill *et al.*, 2004) by searching MS/MS data against the NCBI nr database. Since only partial sequences of these isoforms are described for *M. tardigradum* we found only one peptide (score 51) for Hsp70 isoform 1 and three peptides (protein score of 145) for Hsp70 isoform 2. However, since the ion scores are lower than the selected threshold, these proteins are not regarded as significant and therefore not included in table 1. Interestingly, these two isoforms match to two contigs (protein no. 22, protein no. 24, table 2) from the tardigrade database. The alignment of the partial sequences to contigs is shown in figure 4. It has been shown that both isoforms have specific functions for cryptobiosis (Schill *et al.*, 2004). In addition we found two contigs (protein no. 25, 26, table 2), which are annotated as hypoxia up-regulated protein 1. This protein belongs to the Hsp70 protein family and is described to have a pivotal role in cytoprotective cellular mechanisms triggered by oxygen deprivation. It functions as a molecular chaperone and involves in protein folding (Ozawa *et al.*, 1999).

### **DnaJ protein family**

ATP binding and hydrolysis are essential for the chaperone activity of Hsp70 proteins. The ATPase cycle is controlled by co-chaperones of J-domain proteins by targeting Hsp70s to their substrates and by nucleotide exchange factors, which determine the lifetime of the Hsp70-substrate complex (Mayer & Bukau, 2005). By searching MS/MS data against the tardigrade database we found 13 contigs (protein no. 1-13, table 2) belonging to the DnaJ protein family (Hsp40 family or J family). J proteins are defined by the presence of a J-domain that can regulate the activity of Hsp70 proteins. The sequence-based classification of J proteins suggests the presence of three classes whereby type I J proteins are defined as true homologues of DnaJ (Cheetham & Caplan, 1998). We found DnaJ homologues among all three classes. Figure 5 shows the protein sequence of two members of the DnaJ subfamily A namely DnaJ homologue subfamily A member 1 (Q5NVI9) and DnaJ homologue subfamily A member 2 (Q2HJ94), which are aligned to two contigs from tardigrade protein database, respectively. The J-domain

(indicated in magenta) found in the tardigrade sequences has a compact helical structure with two or three helical domains (indicated with black boxes), which is characteristic for J-domains. It contains also the conserved tripeptide His-Pro-Asp (in green boxes), which is essential for DnaJ-DnaK interactions. The J-domain is linked by a glycine rich region (yellow) to a zinc-finger domain (cysteine-rich (CR)-domain), which is followed by a poorly conserved C-terminal CTD-domain (Linke *et al.*, 2003; Walsh *et al.*, 2004). The CR-domain contains four repeated CXXCXGXG motifs (blue), which are invariable to all class I DnaJ proteins (Bardwell *et al.*, 1986; Linke *et al.*, 2003; Martinez-Yamout *et al.*, 2000). The contigs from tardigrade database exhibit all features of the DnaJ family. There are two other contigs belonging to subfamily A, but since they are much shorter in length we could not find all the regions and domains characteristic for subfamily A: contig fun1\_c5750:1:741:1 represents the C-terminus of subfamily A member 1 and contains only one CXXCXGXG motif; contig fun1\_c14868:431:829:2 contains the conserved tripeptide His-Pro-Asp and a compact helical domain indicating the J-domain. The heat shock proteins DnaJ (Hsp40), DnaK (Hsp70), and GrpE are members of a highly efficient chaperone machinery (Linke *et al.*, 2003). GrpE is a co-chaperone of DnaK and functions as a nucleotide exchange factor. Both co-chaperones GrpE and DnaJ regulate the nucleotide-bound state of DnaK and thus control the flux of unfolded polypeptides into and out of the substrate binding domain of DnaK.

### Chaperonin family

Hsp60 chaperones, also termed chaperonins are composed of two different types of subunits: a 60 kDa protein, known as cpn60 (GroEL in bacteria) and a 10 kDa protein, known as cpn10 (GroES in bacteria) (Ellis & van der Vies, 1991). In general, chaperonins are classified in two different types. Type I chaperonins, such as GroEL, found in eubacteria, mitochondria and chloroplasts form homoheptameric structures and require co-chaperone such as GroES (Cuellar *et al.*, 2008; Walter & Buchner, 2002). The chaperonins of type II are found in archaea and can form octamers or nonamers of 1-3 different subunits and do not require a co-chaperone (Cuellar *et al.*, 2008; Walter & Buchner, 2002). In the present study we identified a chaperonin protein (Hsp60) with high sequence coverage of 70.9% (protein no. 14, table 2). The deduced amino acid sequence of Hsp60 shows the highest homology to *gallus gallus* Hsp60 (similarity of 81.9%, identity 73.9%). The protein sequence of this contig and peptide matches by searching the NCBI nr database are shown in figure 6. Peptide sequences from different species are indicated by different colours.



### Heat shock protein 90 family

Hsp90 as a cytoplasmic stress protein interacts with denatured proteins to prevent irreversible aggregation and degradation. Hsp90 seems to be unusual among heat shock proteins due to its highly selective interaction with regulators of growth, differentiation and apoptosis (Connolly & Hall, 2008). Hsp90s have stress-related as well as housekeeping functions (Mayer & Bukau, 1999) and are shown to be essential for survival in *Drosophila* and yeast (Latchman, 1999). Hsp90s play fundamental roles in signal transduction, cellular trafficking, epigenetic regulation, and morphological and development evolution (Mayer *et al.*, 2009). Among vertebrates there are two Hsp90 isoforms, Hsp90 $\alpha$  and Hsp90 $\beta$ . The database search against NCBI nr and tardigrade databases delivered the identification of both isoforms in *M. tardigradum*. The amino acid sequence of Hsp90 $\beta$  from the tardigrade database is shown in figure 7 and the peptide sequences found in the NCBI nr database are underlined in different colours for different species.

### Small heat shock protein family

Beside the described Hsps we identified heat shock proteins belonging to the family of small heat shock proteins (sHsps). sHsps are homologues of vertebrate alpha-crystallin proteins found in eye lenses and act as molecular chaperones by preventing aggregation of denatured proteins under environmental stress like heat and desiccation (Laksanalamai & Robb, 2004). sHsps have the smallest subunits of all Hsps, with a molecular weight ranging from 15 to 40 kDa. The presence of at least one heat shock-regulated homologue of sHsp in most organisms including extremophiles and the lack of their expression at normal growth temperatures suggests their exigency and importance of sHsps for surviving under stress conditions (Laksanalamai *et al.*, 2001; Roy *et al.*, 1999). The small mammalian heat shock protein is designated as Hsp25 in mouse and as Hsp27 in human. The deduced amino acid sequences of small heat shock proteins found in *M. tardigradum* showed the highest homology to heat shock protein beta 1 (Hsp25) of *Mus musculus* and heat shock protein 67B1 (Hsp67B1) of *Drosophila melanogaster*.

In addition we have found different protein sequences, which have been annotated as major egg antigen. Major egg antigen (alternative name p40) belongs to the small heat shock protein (Hsp20) family and is described in *Schistosoma masoni* (blood fluke, flat worm) for the first time. In particular small heat shock proteins seem to aggregate in response to heat shock and translocate to the nucleus where they appear to be incorporated to the nucleoskeleton and may play a role in stabilizing chromatin (Nene *et al.*, 1986). Structurally, major egg antigen is built of two tandem Hsp20 domains (alpha-crystallin domain (ACD)) in 132-214 aa, 259-346 aa regions and has a molecular weight of 40 kDa. The identified protein contigs from *M. tardigradum* contain only one alpha-crystallin domain. Since tardigrade sequences are derived from an EST

database, it is most likely that just a part of the whole protein of p40 was sequenced. Furthermore the gel slice, in which we found major egg antigen with the highest number of unique peptides, corresponds to a molecular weight of ca. 55 kDa.

In general we found heat shock proteins from slice 8 to slice 18, which indicates the smearing of proteins in the 1D gel probably due to overloading effects. However, the proteins with the highest sequence coverage and queries matches always correspond to the gel slice at the expected molecular weight. For instance Hsp71 with a sequence coverage of 62.1% in the active state (protein no. 18, table 2) is identified predominantly in slice 9 at approximately 70 kDa and Hsp90 $\beta$  (protein no. 28, table2) with a sequence coverage of 61.7% in the active state belongs to slice 8 at approximately 90 kDa. The small heat shock proteins Hsp27 and Hsp67B1 are identified mainly in slice 18 at approximately 28 kDa, which is in accordance with their expected molecular weight.

### **Comparative analysis of heat shock proteins in active versus anhydrobiotic state**

For comparative analysis of heat shock proteins we considered the results of the tardigrade database search as listed in table 2. A total of 46 heat shock proteins were identified including 38 heat shock proteins present in both the active and anhydrobiotic state. Two members of DnaJ family (protein no. 4, 12) and two members of small heat shock proteins (protein no. 34, 36) were exclusively found in the active state whereas four other heat shock proteins (protein no. 6, 7, 9, 46), three of which belong to DnaJ family, could only be identified in the anhydrobiotic state.

The comparative analysis of heat shock proteins in tardigrades in the active *versus* the anhydrobiotic state was performed using label-free technique based on emPAI values to achieve a simple and fast quantification. This method employs normalization by interpreting signals of molecules that do not change concentration from sample to sample. The protein abundance index (PAI) is defined as the number of identified peptides divided by the number of theoretically observable tryptic peptides for each protein, which was later converted to exponentially modified PAI (emPAI, the exponential form of PAI minus one) (Ishihama *et al.*, 2005). The success of using emPAI was demonstrated by determining absolute abundance of 46 proteins in a mouse whole-cell lysate, which had been measured using synthetic peptides (Liu *et al.*, 2004). The emPAI can be directly used for reporting approximate protein abundances in a large-scale analysis as shown in different studies (Barrios-Llerena *et al.*, 2006; Graham *et al.*, 2007; Mann & Mann, 2008; Mann *et al.*, 2008a, 2008b). To facilitate the comparison, we calculated the difference of emPAI (or Sum empAI) of each protein in active and anhydrobiotic state (Supplementary Table 3).

Among the identical heat shock proteins in both states 8 proteins (protein no. 1, 10, 12, 16, 27, 40, 42, and 44) show the same emPAI or Sum emPAI values. The most expressed heat shock protein in the anhydrobiotic state belongs to the Hsp90 family. This is in agreement with the gene expression analysis of a partial *Mt-hsp90* sequence showing up-regulation in the anhydrobiotic state (Reuner *et al.*, 2009). In addition we have identified two members of Hsp90 family that are highly expressed in active animals.

The gene expression analysis of one selected Hsp60 sequence showed no increased expression during anhydrobiosis (Reuner *et al.*, 2009). However, in our data the most abundant Hsp60 protein is up-regulated in active state. Furthermore one hsp60 contig (protein no. 16) has identical emPAI comparing active *versus* tun state, which is in agreement to the gene expression data.

Among the Hsp70 family two heat shock cognate 70 proteins are the most abundant proteins in the active as well as in the anhydrobiotic state. Both of these proteins are down-regulated in the anhydrobiotic state. This supports the results of Jönsson and Schill who detected a lower protein level of Hsp70 in the eutardigrade species *R. coronifer* in anhydrobiotic state using an immunoblot method (Jonsson & Schill, 2007).

By contrast, five protein members of Hsp70 family show slight up-regulation in anhydrobiotic state. This includes two hypoxia up-regulated proteins and Hsp 105kDa, hsc71 and 78 kDa glucose-regulated protein. Schill *et al* (2004) described partial sequences of three isoforms of Hsp70 from *M. tardigradum*. Transcription of isoform 2 was significantly induced in the transitional state II between the anhydrobiotic and active states. As described before we could align the partial sequences of Hsp70 isoform 1 to contig fun1\_c6196:190:2046:1 and Hsp70 isoform 2 to contig fun1\_c166:202:2160:1. The comparison of protein expression level shows that both of these proteins are slightly up-regulated in anhydrobiotic state. Since the antibody used by Jönsson and Schill (2007) is reactive to diverse members of Hsp70 family, they have probably detected an abundant Hsp70 isoform like Hsc70 in *M. tardigradum*. The assumption that *M. tardigradum* and *R. coronifer* share similar molecular mechanism during desiccation, is supported by the result that the most abundant Hsp 70 family member is down-regulated in anhydrobiotic state in both species. The generally low abundance of up-regulated Hsp70 proteins in anhydrobiotic state indicates that these proteins contribute only a small part of the Hsp70 contingent in the cell, which confirms the same hypothesis suggested by Reuner *et al.* (Reuner *et al.*, 2009)

The investigation of sHsp regulation in diapausing organisms indicates a broad range of functions. Different studies revealed the importance of small heat shock protein in diapause and anhydrobiotic animals. In *Sesamia nonagrioides* (Lepidoptera: Noctuidae) two sHps have been suggested to have important roles in the regulation of diapauses. One (SnoHsp19.5) was consistently expressed, while the other one (SnoHsp20.8) was down-regulated in deep

diapause and was up-regulated at the termination of diapause. Furthermore it was shown that up-regulation of Hsps including sHsps during diapause is a major factor contributing to cold-hardiness of overwintering insects. Gene expression studies of some selected heat shock proteins in *M. tardigradum* showed the up-regulation of one sHsp (*Mt-shsp 17.2*) by heat-shock treatment and the down-regulation of *Mt-19.5* in the transition state from the anhydrobiotic to the active state (Reuner *et al.*, 2009). However, the role of these two sHsps in anhydrobiosis is not yet clear. In our investigation the most abundant heat shock protein in tardigrades in the active as well as in the anhydrobiotic state is the small heat shock protein major egg antigen (p40). The importance of sHsps has already been shown in *Artemia franciscana*. The sHsp p26 was extremely accumulated in diapausing embryos (Liang, Amons, Clegg *et al.*, 1997; Liang, Amons, Macrae *et al.*, 1997). The protein p26 is thought to protect the nuclear matrix proteins in cooperation with Hsp70 (Willsie & Clegg, 2002).

### Summary

A complementary approach incorporating genomics and proteomics workflows has resulted in a broad and first-pass coverage of heat shock proteins present in tardigrades in the active and anhydrobiotic state. Mass spectrometric analysis and subsequent database searches yielded proteins of different heat shock protein families. We identified Hsp60, Hsp70, Hsp90 $\alpha$  and Hsp90 $\beta$  by searching MS/MS data against the NCBI nr database as well as the tardigrade database. Even though heat shock proteins are highly conserved; some heat shock proteins were found exclusively by searching MS/MS data against the tardigrade database. Especially different members of DnaJ (Hsp40) family and small heat shock protein family could be identified only by using the tardigrade database. Our proteomic results include heat shock proteins, which are described to the best of our knowledge for the first time in *M. tardigradum*. The small heat shock protein major egg antigen (p40), which is the most abundant protein in both states, is one of these proteins.

Using emPAI we performed a comparative analysis of the expression of heat shock proteins in the active *versus* the anhydrobiotic state. Our study provides additional data towards understanding the heat shock protein expression during anhydrobiosis. In parallel to the gene expression analysis of heat shock proteins in *M. tardigradum* we found a member of Hsp90 family up-regulated in the anhydrobiotic state. Furthermore the most abundant protein members of Hsp70 family are down-regulated in the anhydrobiotic state, which is in agreement with the observations in *R. coronifer*. Since up-regulated Hsp70 proteins in the anhydrobiotic state are generally low abundant, we conclude that they contribute only a small part of the Hsp70 contingent in the cell.

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## Secondary Summary

### Zusammenfassung

Tardigraden haben die außergewöhnliche Fähigkeit, extreme Stress-Bedingungen zu überleben, indem sie Kryptobiose eingehen. Dabei ändern sie ihre Körperform, ziehen sich zusammen und bilden ein Tönnchen, in dem keinerlei Metabolismus mehr nachweisbar ist. Obwohl Tardigraden seit ca. 300 Jahren bekannt sind, sind die molekularen Mechanismen während der Kryptobiose aufgrund fehlender fundamentaler Untersuchungen noch unklar. Eine wichtige Proteinklasse, die beim Überleben der Tardigraden während der Stress-Situation möglicherweise eine große Rolle spielt, sind die Hitzeschockproteine. Hitzeschockproteine (Stressproteine, Hsps) sind ubiquitäre, hoch konservierte Chaperone, die die Aufrechterhaltung der Proteine in ihrer funktionellen Konformation bewirken, indem sie ihre Aggregation verhindern. Während die Anwesenheit der Hitzeschockproteine in der Zelle unter normalen Bedingungen erforderlich ist, ist ihre Aktivität bei Hitze oder in Stress-Situationen zum Überleben absolut notwendig. In unserer Studie haben wir die Hitzeschockproteine in *Milnesium tardigradum* unter Verwendung von Proteomics-Techniken identifiziert. Dazu wurde die eindimensionale Gelelektrophorese gefolgt von der hochsensitiven Analyse der nach Trypsin-Verdau erhaltenen Peptide durch nanoLC-ESI-MS/MS auf einem LTQ-Orbitrap-Massenspektrometer eingesetzt. Proteine aus diversen Hitzeschock-Proteinfamilien Hsp40, Hsp60, Hsp70 und Hsp90 sowie kleine Hitzeschockproteine (Hsp20) und zusätzlich Co-Chaperone wie GrpE konnten durch MS/MS Analyse und Datenbank-Suche gegen die NCBI- sowie eine neu zusammengestellte Proteindatenbank aus EST-Sequenzen von *Milnesium tardigradum* nachgewiesen werden. Außerdem konnten durch eine semiquantitative Methode die identifizierten Hitzeschockproteine in Tardigraden im aktiven und anhydrobiotischen Zustand verglichen werden.

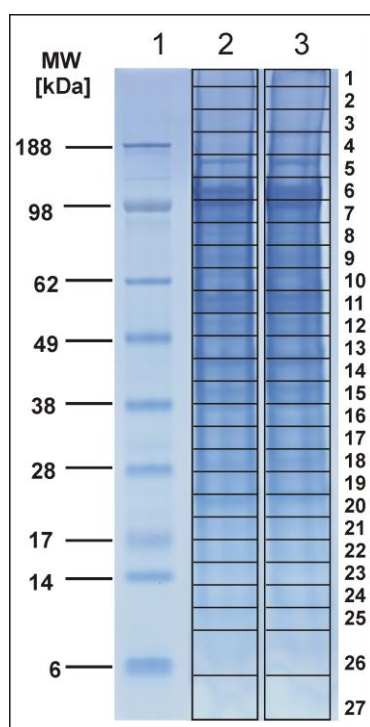
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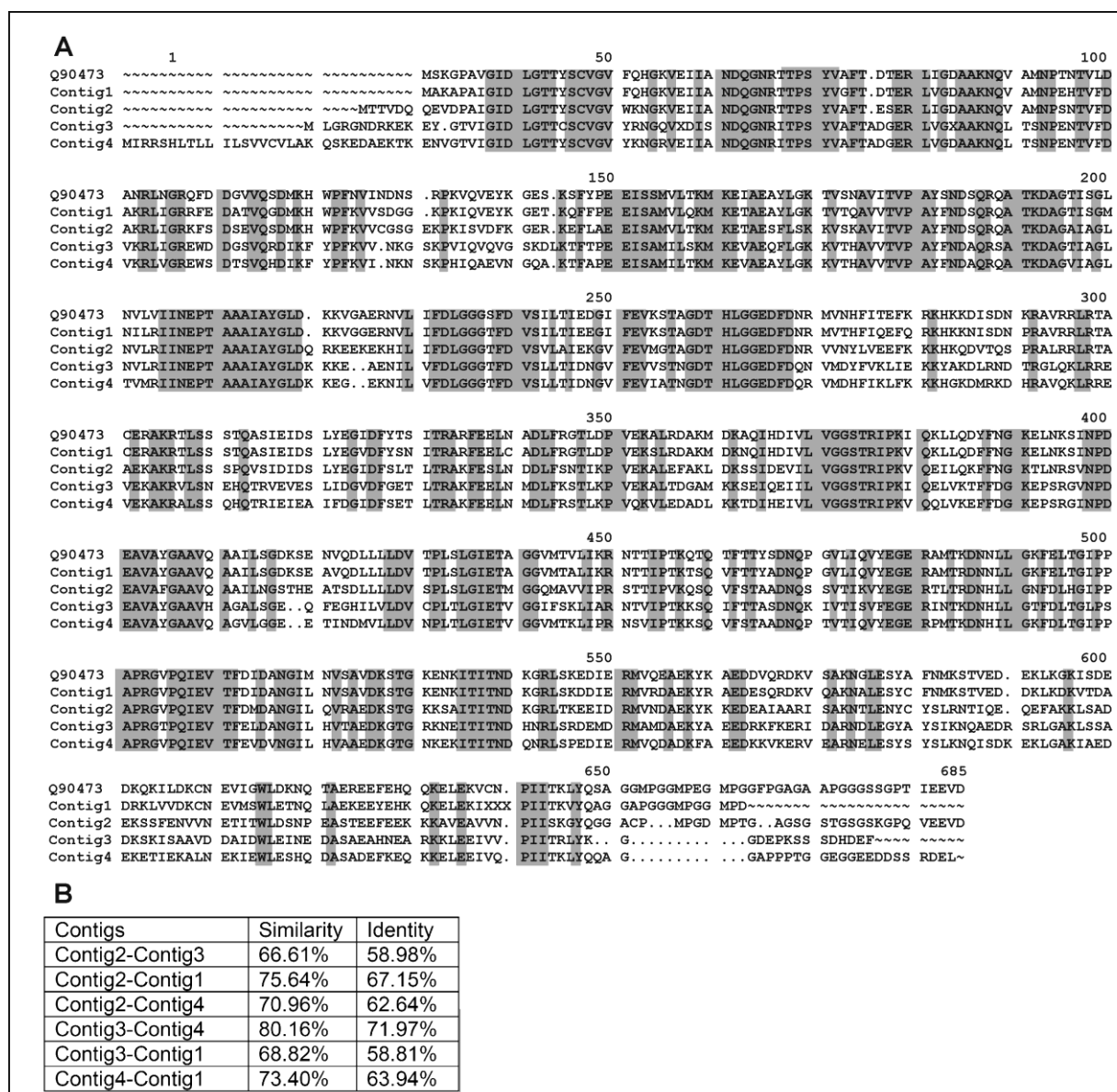
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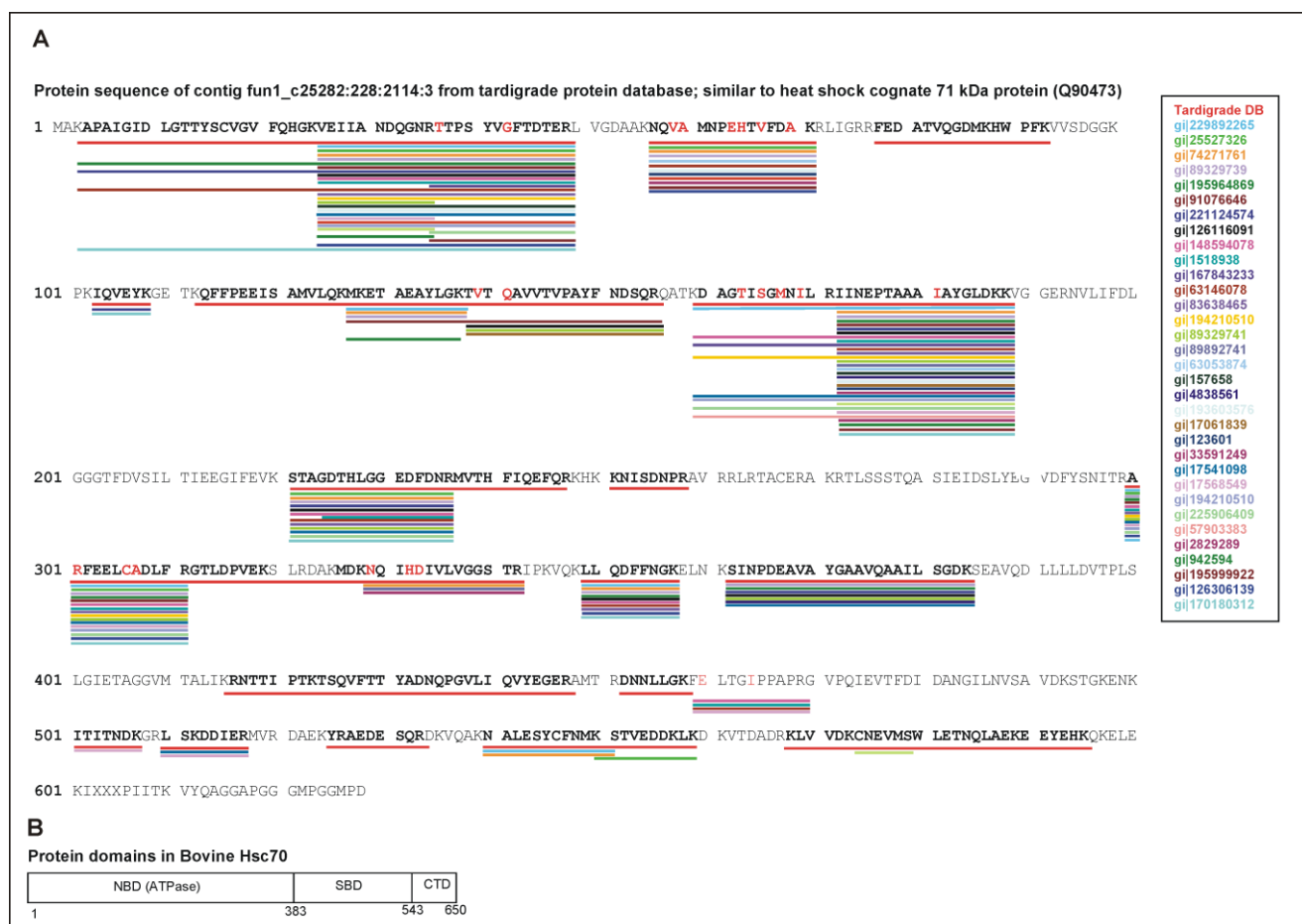
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**Figures**

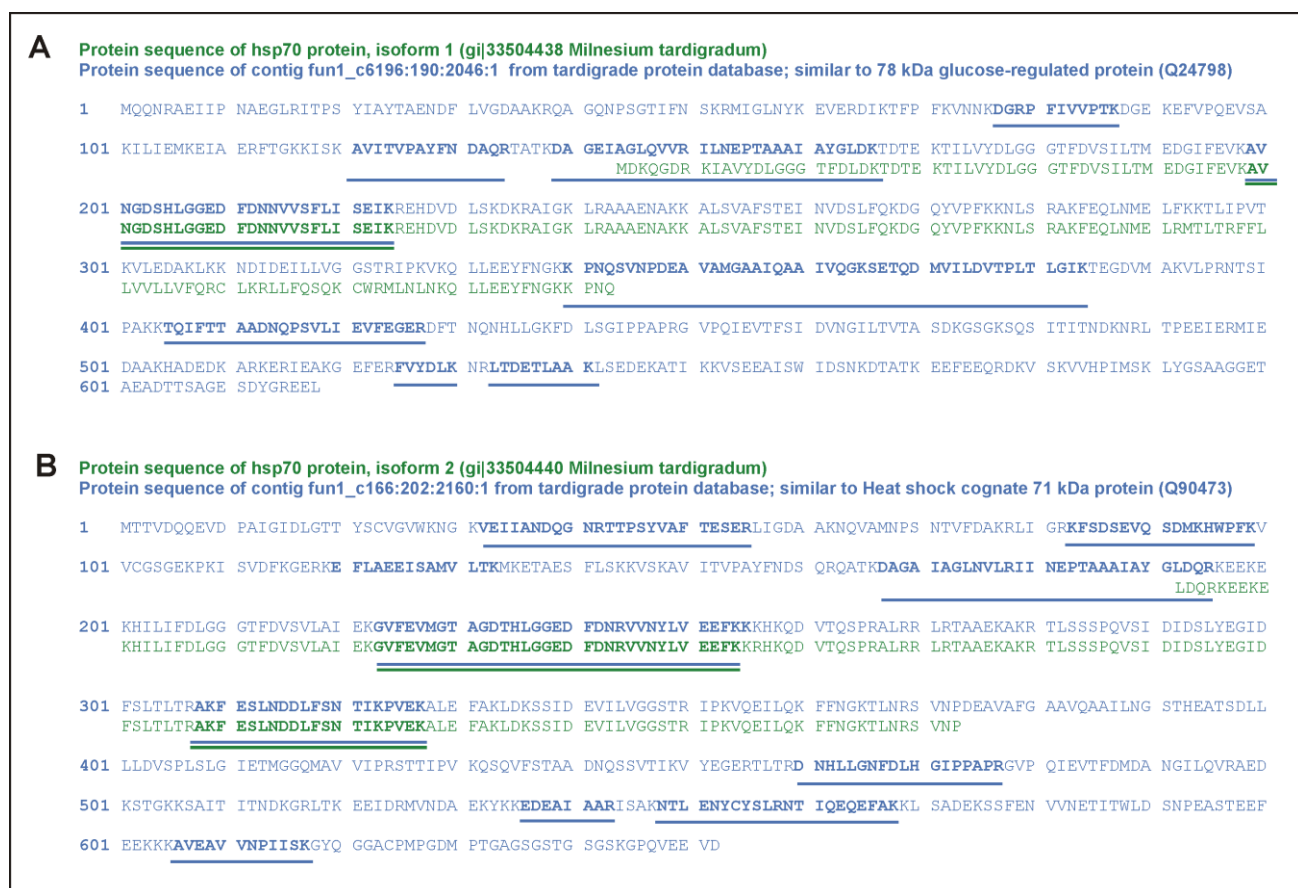
**Figure 1: Electrophoretic separation of protein extract of tardigrades in active and anhydrobiotic states on a one-dimensional polyacrylamide gel.** Lane 1: Rainbow molecular weight marker. Lane 2: Protein extract of whole tardigrades in the active state. Lane 3: Protein extract of whole tardigrades in the anhydrobiotic state. The sections cut out for in-gel digestion are numbered 1-27.



**Figure 2: Multiple alignment of Hsp70 isoforms similar to Hsp71 (*Danio rerio* Q90473). A:** Four contigs, which show the highest homology to Hsp71 (*Danio rerio*) are aligned. Identical amino acids in all five sequences are highlighted. **B:** The similarity and identity between the contigs are listed. Contig1: fun1\_c25282:228:2114:3 (no. 18, table 2), contig2: fun1\_c166:202:2160:1 (no. 22, table 2), contig3: fun1\_c2462:685:2595:1 (no. 21, table 2), contig4: fun1\_c3361:227:2224:2 (no. 17, table 2). The sequences of all contigs are given in Supplementary Table 2.



**Figure 3: Alignment of Hsp70 sequence of *M. tardigradum* to identified peptide sequences in the NCBI nr database.** The peptide sequences found by MS/MS search against the tardigrade database are in bold and underlined in red. Sequences with more than two amino acid substitutions are marked in red. The identified peptide sequences from different species present in the NCBI nr database are underlined using different colours for every protein hit. Corresponding gi-accession numbers are listed on the right in the same colour.



**Figure 4: Alignment of partial sequences of *M. tardigradum* Hsp70 isoform 1 and 2 as obtained from the NCBI nr database to the corresponding sequences found in the tardigrade database. Partial sequences of Hsp70 isoforms 1 (A) and 2 (B, green) are aligned to the corresponding sequences from tardigrade database (blue). Identified peptides are in bold and underlined.**

## A

## Subfamily A member 1 (Contig1: fun1\_c28015:327:1157:3)

(Q5NVI9) 1 MVKETTYYDV LGVKPNATQE ELKKAYRKLAKYHPDKNPN E..GEKFKQI SQAYEVLSDA KKRELYDRGG  
 (Contig1) MVRETGLYDE LGVKSTAADD ELKKAYRKLAKYHPDKNPD PAAEKFKRI SFAYEVLSDQ KKREMYDRGG

(Q5NVI9) 71 EQAIKEGGAG GGFSGSPMDIF DMFFGGGR.. MQRERRGKN VVHQLSVTLE DLYNGATRKL ALQKNVICDK  
 (Contig1) EQALKEGGGG ADFHHAQDIF DMFFGFGGGR GAQRERKGD VVHQLKVTLE DLYNGCSRRL ALQKSVICKK

(Q5NVI9) 141 CEGRGGKK.G AVECCPNCRG TGMQIRIHQI GPGMVQQIQS VCMECQGHGE RISPKDRCKS CNGRKIVREK  
 (Contig1) CEGRGCKKGA SEMQCPGCRG QGMKIRTMQL APGFVQQSQS ICNDCGGGQ RIKPEDRCPD CEGKVVVRR

(Q5NVI9) 211 KILEVHIDKG MKDGQKIFH GEGDQEPGLE PGDIIIVLDQ KDHAVFTRRG EDLFCMDIQ LVEALCGFQK  
 (Contig1) KILEVHVDKG MKDGQKIMFT GEGDQEPGLQ PGDVYIVLDE QEHEEFKRRG NDLVMQMNIQ LVXSAV

(Q5NVI9) 281 PISTLDNRTI VITSHPGQIV KHGDIKCVLN EGMPIYRRPY EKGRLLIEFK VNFPENGFLS PDKLSLLEKL

(Q5NVI9) 351 LPERKEVEET DEMDQVELVD FDPNQERRRH YNGEAYEDE HHPRGVQCQ TS

## B

## Subfamily A member 2 (Contig2: fun1\_c4406:232:1473:1)

(Q2HJ94) 1 MANVADTKLY DILGVPPGAS ENELKKAYRK LAKEYHPDKN PNAGDRFKETI SFAYEVLNSP EKRELYDRYG  
 (Contig2) ...MPDSKLY DILGVQRNAS ENELKKAYRK LAKEYHPDKN PEEGEKFKETI SFAHEILTDP QKRSLYDRGG

(Q2HJ94) 71 EQGLREGSGG GGMDDIFSH IFGGG..LF SFMGNQSRSR NGRRRGEDMM HPLKVSLEDL YNGKTKLQL  
 (Contig2) LDLSLKEG.GM GHDMEDILGS MFGGGGGGLF SSFFGGGMGG RRRQRGEDITV FPMNVTLEDL YKGTAKISI

(Q2HJ94) 141 SKNVLCSACS GGGKSG.AV QKCSACRGRG VRIMIRQLAP GMVQQMQSVC SDCNGEGEVI NEKDRCKKCE  
 (Contig2) EKTIIICPSCN GTGGKSGKLA NRCSTCRGTG VKVSLRPLGP GMMQQIQEEC GKCRGTGQEF SDSDRCNKCD

(Q2HJ94) 211 GKKVIKEVKI LEVHVDKGMK HGQRITFTGE ADQAPGVEPGD IVLLLQEK HEVFQRDNGD LHMTYKIGLV  
 (Contig2) GKQVVRDKKV VDVHIDKGRR STDRIILRGE GDQKPGIESGD VIVLLQQLK HEVFEREGEH LFMTMTIDL

(Q2HJ94) 281 EALCGFQFTF KHLDGRQIVV KYPPGKVI EP GCVRVVRGEGM PQYRNPF EK GDLYIKFDVQ FPENWIN.P  
 (Contig2) DALCGFQRTI THLDGRLLI KRGPGEVTEP .KIYTVVGEEM PVYRNPMVK GNLMIKFTVD FPSDSWKPN

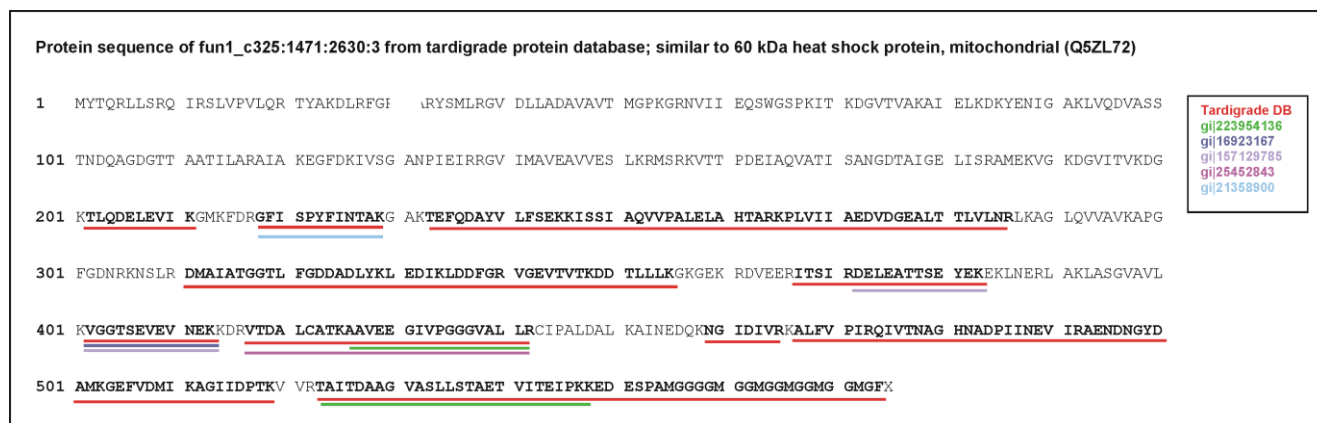
(Q2HJ94) 351 DKLSELEDL PSRPEVPNII GDTEEVELQE FDSTRGSGGGQ RREAYNDSSD EESSHHGP G.VQCAHQ  
 (Contig2) DVLKHLEELL GPRPTPPKIA NTEEVVEVSL HSLQGHSEGR ESSSRGEAYD EDDEESEGP GNVRCQSQ

## C

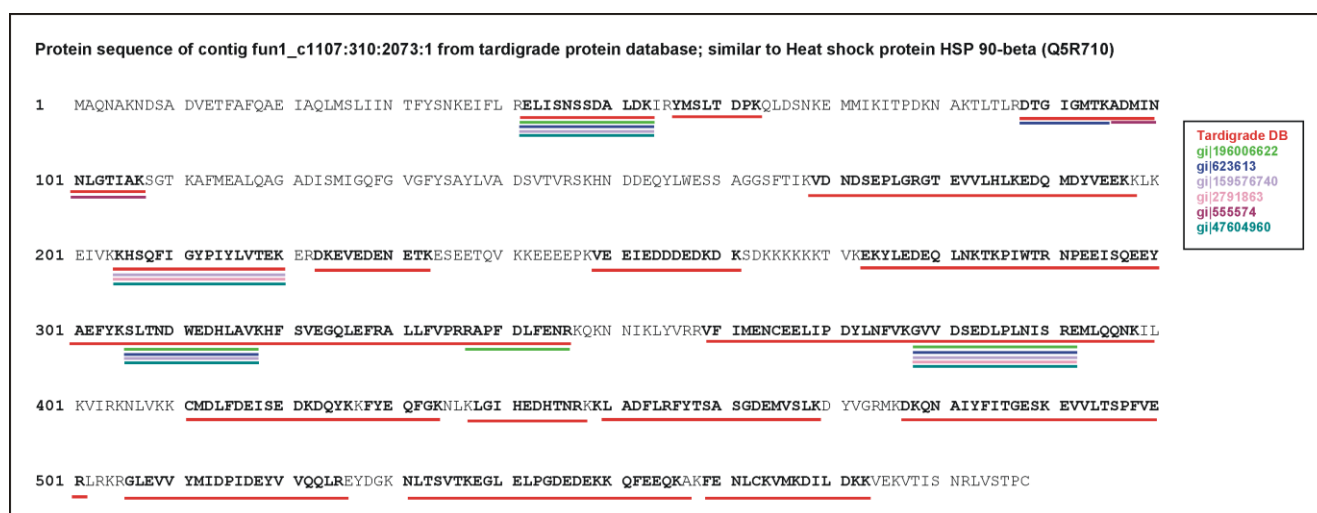
Protein sequence	Residue of J-domain	Residues of helical region in J-domain	Residues of CXXCXGXG motifs in zinc binding CR-domain
Subfamily A member 1 Q5NVI9	6-68	17-33, 43-66	133-140, 149-156, 176-183, 192-199
Contig1	8-70	15-32, 43-68	138-145, 155-162, 182-189, 198-205
Subfamily A member 2 Q2HJ94	4-70	21-34, 47-55, 63-69	143-150, 159-166, 186-193, 202-209
Contig2	1-70	14-31, 41-53	142-149, 159-166, 186-193, 202-209

**Figure 5: Analysis of DnaJ subfamily A member 1 and member 2.** The protein sequences of DnaJ subfamily member 1 (A, contig1) and member 2 (B, contig2) are aligned to the corresponding protein sequences (Q5NVI9, Q2HJ94) found by homology search, respectively. Characteristic domains for J proteins like J-domain (magenta), CXXCXGXG motifs in the zinc

binding CR-domain (blue) and glycine-rich domains (yellow) are compared and highlighted. Furthermore important features for J-domains are shown: helical features (in black boxes) and the conserved tripeptide His-Pro-Asp (in green box). C: The residues of J-domain, helical region and CXXCXGXG motifs in the zinc binding CR-domain are listed.



**Figure 6: Alignment of Hsp60 sequence of *M. tardigradum* to identified peptide sequences in the NCBI database.** The peptide sequences found by MS/MS search against the tardigrade database are in bold and underlined in red. The identified peptide sequences from different species in the NCBI database are underlined using different colours for every protein hit. Corresponding gi-accession numbers are listed on the right in the same colour.



**Figure 7: Alignment of Hsp90-beta sequence of *M. tardigradum* to identified peptide sequences in the NCBI database.** The peptide sequences found by database search against the tardigrade database are in bold and underlined in red. The identified peptide sequences from different species in the NCBI database are underlined using different colours for every protein hit. Corresponding gi-accession numbers are listed on the right in the same colour.

**Table 1: Heat shock proteins identified in *M. tardigradum* using the NCBIInr database.**

Acquired MS/MS data were searched against the NCBIInr database. Protein number, slice number (A indicates active state and T tun (anhydrobiotic) state), protein annotation, accession number, total protein score, number of matched peptides, sequence coverage, protein mass, and emPAI (Sum emPAI) are listed. Identical proteins identified in different slices are listed only once and the spot with the highest protein score (in bold) is ranked at the top. Sum emPAI is calculated in the case that the same protein was found more than once in a slice.

**Table 2: Heat shock proteins identified in *M. tardigradum* using the tardigrade protein database.**

Acquired MS/MS data were searched against the NCBIInr database. Protein number, slice number (A indicates active state and T tun (anhydrobiotic) state), protein annotation, total protein score, number of matched peptides, sequence coverage, protein mass, and emPAI (Sum emPAI) are listed. Identical proteins identified in different slices are listed only once and the spot with the highest protein score (in bold) is ranked at the top. Sum emPAI is calculated in the case that the same protein was found more than once in a slice.



Table 1: Heat shock proteins identified in *M. tardigradum* using the NCBI nr database.

Protein no.	State/slice no.	Accession no.	Protein description	Protein Score	Protein mass	Significant unique peptides	Protein coverage (%)	Protein pI	emPAI	Sum emPAI
<b>Chaperonin (Hsp60) family</b>										
1	A10	gi 150398979	chaperonin GroEL [Methanococcus vannielii SB]	87	57303	1	3.2	5.21		
	T10	gi 150398979	chaperonin GroEL [Methanococcus vannielii SB]	90	57303	1	3.2	5.21		
2	A10	gi 16923167	heat shock protein 60 [Salmo salar]	88	17453	1	7.2	5.15		
	T10	gi 16923167	heat shock protein 60 [Salmo salar]	87	17453	1	7.2	5.15	0.19	
3	A10, 9-16	gi 223954136	heat shock protein 60 [Litopenaeus vannamei]	279	61015	2	10.9	5.84	0.17	0.76
	T10, 9-17	gi 223954136	heat shock protein 60 [Litopenaeus vannamei]	227	61015	3	9.3	5.84	0.23	0.81
4	A10	gi 157129785	chaperonin-60kD, ch60 [Aedes aegypti]	247	61155	1	7	5.47	0.11	
5	A10, 14	gi 197102016	60 kDa heat shock protein, mitochondrial precursor [Pongo abelii]	197	61130	1	4.4	5.7	0.11	0.22
6	T10	gi 197700758	chaperonin-60 [uncultured bacterium]	76	19938	1	8.2	4.65	0.17	
7	T10	gi 21358900	60 kDa chaperonin [uncultured pig faeces bacterium]	77	20206	1	7	4.83	0.17	
8	T10	gi 5912574	Chaperonin [Paracentrotus lividus]	122	62195	1	3.4	5.12	0.05	
9	T10	gi 25452843	RecName: Full=60 kDa chaperonin	121	57518	2	4	5.76	0.12	
<b>Heat shock protein 70 family</b>										
10	A9	gi 126116091	heat shock protein 70 cognate [Silurus meridionalis]	663	71072	7	17.6	5.37	0.5	
	T9	gi 126116091	heat shock protein 70 cognate [Silurus meridionalis]	714	71072	8	17.6	5.37	0.64	
11	A9	gi 126306139	PREDICTED: similar to heat shock protein 70 [Monodelphis domestica]	419	70887	5	12.4	5.95	0.31	
	T9	gi 126306139	PREDICTED: similar to heat shock protein 70 [Monodelphis domestica]	455	70887	6	9.3	5.95	0.31	
12	A9	gi 156361178	predicted protein [Nematostella vectensis]	556	73993	6	10.8	5.32	0.54	
	T9	gi 156361178	predicted protein [Nematostella vectensis]	466	73993	5	9.6	5.32	0.48	
13	A9, 8	gi 167843233	heat shock protein 70 precursor [Tigriopus japonicus]	304	76137	4	8.1	5.66	0.18	0.27
	T9, 8	gi 167843233	heat shock protein 70 precursor [Tigriopus japonicus]	338	76137	4	8.7	5.66	0.18	0.31
14	A16	gi 157658	heat shock protein cognate 72 [Drosophila melanogaster]	402	72304	4	11.7	5.22	0.31	
	T8, 10	gi 157658	heat shock protein cognate 72 [Drosophila melanogaster]	164	72304	1	6.2	5.22	0.09	0.18
15	A9	gi 170180312	heat shock protein 70 [Moina macrocopa]	662	71278	6	16.5	5.37	0.43	
	T9	gi 170180312	heat shock protein 70 [Moina macrocopa]	718	71278	7	17.4	5.37	0.57	
16	A9	gi 17541098	Heat Shock Protein family member (hsp-1) [Caenorhabditis	713	69965	6	16.7	5.44	0.44	

			elegans]								
	<b>T9</b>	gij17541098	Heat Shock Protein family member (hsp-1) [Caenorhabditis elegans]	791	69965	7	18.6	5.44	0.66		
<b>17</b>	<b>A9</b>	gij17568549	Heat Shock Protein family member (hsp-3) [Caenorhabditis elegans]	587	73093	7	14.4	4.95	0.55		
	<b>T9</b>	gij17568549	Heat Shock Protein family member (hsp-3) [Caenorhabditis elegans]	540	73093	5	10.3	4.95	0.49		
<b>18</b>	<b>A9, 13, 17</b>	gij193603576	PREDICTED: similar to heat shock cognate 70 protein isoform 2 [Acyrtosiphon pisum]	643	71626	8	15.1	5.34	0.57	1.02	
	<b>T14</b>	gij193603576	PREDICTED: similar to heat shock cognate 70 protein isoform 2 [Acyrtosiphon pisum]	237	71626	2	8.4	5.34	0.09		
<b>19</b>	<b>A9</b>	gij194210510	PREDICTED: similar to heat shock protein 70 [Equus caballus]	327	71237	4	7.9	5.85	0.2		
	<b>T9</b>	gij194210510	PREDICTED: similar to heat shock protein 70 [Equus caballus]	453	71237	5	10.1	5.85	0.31		
<b>20</b>	<b>A9</b>	gij194384180	unnamed protein product [Homo sapiens]	442	55158	4	13	5.2	0.42		
	<b>T9</b>	gij194384180	unnamed protein product [Homo sapiens]	508	55158	4	15.4	5.2	0.5		
<b>21</b>	<b>A9</b>	gij195964869	heat shock protein 70-1 [Tetranychus cinnabarinus]	755	71522	8	19.6	5.37	0.64		
	<b>T9, 12</b>	gij195964869	heat shock protein 70-1 [Tetranychus cinnabarinus]	827	71522	9	19.9	5.37	0.79	1.04	
<b>22</b>	<b>A9</b>	gij229892265	heat shock protein Hsp70Ab-like [Apis mellifera]	887	70687	11	22.5	5.61	0.89		
	<b>T9</b>	gij229892265	heat shock protein Hsp70Ab-like [Apis mellifera]	906	70687	10	20.9	5.61	0.8		
<b>23</b>	<b>A9</b>	gij57903383	heat shock protein 70 [Heterocapsa triquetra]	224	24815	1	13.1	5.84	0.29		
	<b>T9</b>	gij57903383	heat shock protein 70 [Heterocapsa triquetra]	260	24815	3	13.1	5.84	0.66		
<b>24</b>	<b>A9, 8-10, 12-19</b>	gij195999922	hypothetical protein TRIADDRAFT_63625 [Trichoplax adhaerens]	661	73413	7	13.1	5.25	0.48	2.24	
	<b>T9</b>	gij195999922	hypothetical protein TRIADDRAFT_63625 [Trichoplax adhaerens]	629	73413	6	13.1	5.25	0.48		
<b>25</b>	<b>A9</b>	gij219938539	heat shock protein 70 kDa [Dromia personata]	547	70095	7	14.6	5.4	0.44		
	<b>T9</b>	gij219938539	heat shock protein 70 kDa [Dromia personata]	564	70095	7	14.6	5.4	0.44		
<b>26</b>	<b>A9</b>	gij223036830	heat shock protein 70 [Spodoptera exigua]	364	75041	5	10.8	5.75	0.24		
	<b>T9</b>	gij223036830	heat shock protein 70 [Spodoptera exigua]	314	75041	3	9.3	5.75	0.19		
<b>27</b>	<b>A9, 8, 12, 13</b>	gij89329739	heat shock protein 70 [Capsaspora owczarzaki]	821	53525	8	24.5	5.57	0.93	1.98	
	<b>T9</b>	gij89329739	heat shock protein 70 [Capsaspora owczarzaki]	875	53525	9	27.6	5.57	1.05		
<b>28</b>	<b>A9, 10</b>	gij89329741	heat shock protein 70 [Sphaeroforma arctica]	640	52662	5	21.1	5.27	0.63	0.83	
	<b>T9</b>	gij89329741	heat shock protein 70 [Sphaeroforma arctica]	640	52662	7	19.6	5.27	0.84		
<b>29</b>	<b>A9, 8, 11-13</b>	gij89892741	heat shock protein 70 [Liriomyza huidobrensis]	669	71151	8	13.6	5.47	0.5	1.4	
	<b>T9, 12, 16</b>	gij89892741	heat shock protein 70 [Liriomyza huidobrensis]	610	71151	6	15	5.47	0.5	0.9	

<b>30</b>	<b>A9</b> , 11, 14	gij91076646	PREDICTED: similar to heat shock 70 kD protein cognate [Tribolium castaneum]	735	63256	8	16.5	5.45	0.93	1.25
	<b>T9</b>	gij91076646	PREDICTED: similar to heat shock 70 kD protein cognate [Tribolium castaneum]	744	63256	10	16.5	5.45	0.93	
<b>31</b>	<b>A9</b> , 8	gij63146078	HSP70 [Oxyuranus scutellatus scutellatus]	768	70171	9	21.3	5.66	0.65	0.85
	<b>T9</b>	gij63146078	HSP70 [Oxyuranus scutellatus scutellatus]	780	70171	9	21.3	5.66	0.73	
<b>32</b>	<b>A9</b> , 8-10	gij260816519	hypothetical protein BRAFLDRAFT_114843 [Branchiostoma floridae]	603	72279	6	14.2	5.2	0.43	0.82
	<b>T9</b>	gij260816519	hypothetical protein BRAFLDRAFT_114843 [Branchiostoma floridae]	583	72279	7	13.2	5.2	0.56	
<b>33</b>	<b>A9</b>	gij241119385	Hsp70, putative [Ixodes scapularis]	362	72701	5	10	5.47	0.25	
	<b>T9</b>	gij241119385	Hsp70, putative [Ixodes scapularis]	304	72701	3	8.5	5.47	0.19	
<b>34</b>	<b>A9</b>	gij33591249	heat shock protein 68 [Drosophila erecta]	569	68634	6	13.4	5.85	0.45	
	<b>T11</b>	gij33591249	heat shock protein 68 [Drosophila erecta]	159	68634	2	4.8	5.85	0.1	
<b>35</b>	<b>A16</b> , 17	gij942594	heat shock cognate protein [Urechis caupo]	315	71882	4	8.4	5.19	0.25	0.39
	<b>T16</b>	gij942594	heat shock cognate protein [Urechis caupo]	215	71882	3	8.2	5.19	0.2	
<b>36</b>	<b>A9</b>	gij74271761	heat shock cognate protein 70 [Chilo suppressalis]	833	71553	11	22.4	5.24	0.87	
	<b>T9</b>	gij74271761	heat shock cognate protein 70 [Chilo suppressalis]	830	71553	10	19	5.24	0.71	
<b>37</b>	<b>A8</b>	gij25527326	heat-shock protein 70 [Cotesia rubecula]	265	71784	2	9.3	5.38	0.14	
	<b>T8</b>	gij25527326	heat-shock protein 70 [Cotesia rubecula]	497	71784	5	12.7	5.38	0.31	
<b>38</b>	<b>A9</b>	gij147906703	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) [Xenopus laevis]	726	72489	8	16.2	5.03	0.78	
<b>39</b>	<b>A14</b>	gij157278567	heat shock protein 70 isoform 5 [Oryzias latipes]	174	70682	2	6.7	5.28	0.1	
<b>40</b>	<b>A18</b>	gij123592	RecName: Full=Heat shock 70 kDa protein	154	56728	1	5.2	6.45		
<b>41</b>	<b>A13</b>	gij157679184	HSP70 protein [Poecilia reticulata]	293	69952	2	11.4	5.13	0.15	
<b>42</b>	<b>A10</b>	gij1661112	heat shock 70kDa protein [Mesocestoides corti]	207	70925	1	8.7	5.47	0.09	
<b>43</b>	<b>A9</b>	gij195964871	heat shock protein 70-2 [Tetranychus cinnabarinus]	743	73797	6	16.2	5.26	0.69	
<b>44</b>	<b>A10</b>	gij91242966	heat shock protein Hsp70-9 [Blastocladiella emersonii]	122	71142	1	4.1	5.68		
<b>45</b>	<b>A12</b>	gij62526643	heat shock protein 70 [Culex pipiens]	270	70761	4	8.8	5.55	0.2	
<b>46</b>	<b>A8</b>	gij417153	RecName: Full=Heat shock protein 82	169	77426	1	4.4	4.88	0.09	
<b>47</b>	<b>A11</b>	gij4235279	heat shock protein 70 [Setaria digitata]	305	70477	3	11.8	5.63	0.2	
<b>48</b>	<b>A16</b>	gij61105572	HSP70 [Palaemonetes pugio]	227	11830	1	30.3	5.32	1.14	
<b>49</b>	<b>A15</b>	gij157665	heat shock cognate 4 [Drosophila melanogaster]	235	71300	3	8.3	5.36		

50	T9	gi 148594078	heat shock protein 70 kDa [Cyanagraea praedator]	770	69932	9	17	5.41	0.66	
51	T9	gi 1518938	heat shock protein 70 [Botryllus schlosseri]	597	69653	7	12.2	5.41	0.51	
52	T15	gi 123601	RecName: Full=Heat shock 70 kDa protein	160	71291	2	4.8	5.37	0.09	
53	T11	gi 17061839	stress protein HSC70 [Xiphophorus maculatus]	158	70865	2	5.3	5.44	0.09	
54	T9	gi 171673209	heat shock protein 70 [Phascolosoma esculenta]	726	71778	7	16.4	5.15	0.56	
55	T9	gi 221124574	PREDICTED: similar to heat shock protein 70 [Hydra magnipapillata]	642	72293	6	16.3	5.55	0.56	
56	T10, 8	gi 63053874	heat shock protein 70 [Delia antiqua]	248	71195	3	7	5.48	0.14	0.28
57	T9	gi 256079979	heat shock protein 70 [Schistosoma mansoni]	579	71479	7	11.3	5.12	0.5	
58	T18	gi 2829289	HSP70 [Schistosoma japonicum]	137	71613	2	4.8	5.12	0.09	
59	T9	gi 83638465	heat shock cognate 70 [Tetranychus urticae]	724	71516	9	15.1	5.52	0.64	
60	T9	gi 225906409	heat shock inducible protein 70 [Haliotis diversicolor]	611	70356	6	15.4	5.41	0.51	
61	T10	gi 4838561	heat shock protein 70 [Crassostrea gigas]	253	72226	2	8.2	5.38	0.19	
<b>Heat shock protein 90 family</b>										
62	A10, 5, 6	gi 123681	RecName: Full=Heat shock protein HSP 90-beta	103	83615	1	3	4.97		
	T21	gi 123681	RecName: Full=Heat shock protein HSP 90-beta	119	83615	1	3	4.97	0.04	
63	A14	gi 156544504	PREDICTED: similar to heat shock protein 90 [Nasonia vitripennis]	150	82441	1	5.7	4.98	0.2	
	T8	gi 156544504	PREDICTED: similar to heat shock protein 90 [Nasonia vitripennis]	300	82441	3	7.6	4.98	0.17	
64	A13, 12	gi 159576740	cytosolic heat shock protein 90 alpha [Solea senegalensis]	184	83827	3	5.5	5.04	0.12	0.29
	T8, 9	gi 159576740	cytosolic heat shock protein 90 alpha [Solea senegalensis]	322	83827	4	7.5	5.04	0.17	0.34
65	A15	gi 2791863	heat shock protein 90-beta [Danio rerio]	95	83763	1	4	4.9	0.04	
	T10	gi 2791863	heat shock protein 90-beta [Danio rerio]	130	83763	2	4	4.9	0.08	
66	A8	gi 555574	heat shock protein 90 [Danio rerio]	58	10950	1	11.9	4.79	0.73	
	T12, 13	gi 555574	heat shock protein 90 [Danio rerio]	67	10950	1	11.9	4.79	0.31	0.61
67	A8	gi 623613	heat shock protein 90 [Schizosaccharomyces pombe]	362	80896	4	8.2	4.94	0.17	
	T8	gi 623613	heat shock protein 90 [Schizosaccharomyces pombe]	278	80896	2	6.7	4.94	0.17	
68	A8, 7-9, 11, 12, 16	gi 47604960	heat shock cognate protein HSP 90-beta [Gallus gallus]	543	83717	6	14.2	4.95	0.26	1.05
	T8, 11, 16	gi 47604960	heat shock cognate protein HSP 90-beta [Gallus gallus]	292	83717	3	7.4	4.95	0.17	0.37
69	A10, 9	gi 63509	hsp 108 [Gallus gallus]	101	91446	1	3.3	4.81	0.07	0.11
	T11, 7, 10	gi 63509	hsp 108 [Gallus gallus]	150	91446	2	3.3	4.81	0.07	0.18
70	A11, 7	gi 113931560	heat shock protein 90kDa beta (Grp94), member 1 [Xenopus]	208	92763	3	4.2	4.76	0.11	0.18

			( <i>Silurana</i> ) <i>tropicalis</i> ]							
<b>71</b>	<b>A11</b>	gij183178947	82 kDa heat shock protein [ <i>Philodina roseola</i> ]	172	84899	2	4.9	5.09	0.08	
<b>72</b>	<b>T8</b>	gij115726886	PREDICTED: similar to heat shock 90 kDa protein, partial [ <i>Strongylocentrotus purpuratus</i> ]	297	57815	3	9.1	4.99	0.18	
<b>73</b>	<b>T8</b>	gij194245631	heat shock protein 90 [ <i>Laternula elliptica</i> ]	348	83839	3	7.8	4.86	0.17	
<b>74</b>	<b>T8</b>	gij196006622	Hsp90 [ <i>Trichoplax adhaerens</i> ]	353	83671	3	8.3	4.88	0.21	
<b>Others</b>										
<b>75</b>	<b>A10</b>	gij198420705	PREDICTED: similar to chaperonin containing TCP1, subunit 4 (delta) [ <i>Ciona intestinalis</i> ]	186	58349	2	7.6	8.4	0.12	
	<b>T10, 11</b>	gij198420705	PREDICTED: similar to chaperonin containing TCP1, subunit 4 (delta) [ <i>Ciona intestinalis</i> ]	126	58349	1	5.8	8.4	0.06	0.12
<b>76</b>	<b>A13, 11, 15</b>	gij17564182	Chaperonin Containing TCP-1 family member (cct-7) [ <i>Caenorhabditis elegans</i> ]	67	58962	1	2.6	6.07	0.06	0.12
	<b>T10, 11</b>	gij17564182	Chaperonin Containing TCP-1 family member (cct-7) [ <i>Caenorhabditis elegans</i> ]	102	58962	1	2.6	6.07	0.06	0.12
<b>77</b>	<b>A10</b>	gij969094	chaperonin containing T-complex protein gamma subunit-like protein [ <i>Drosophila melanogaster</i> ]	147	58533	2	3.4	6.38	0.06	
	<b>T10</b>	gij969094	chaperonin containing T-complex protein gamma subunit-like protein [ <i>Drosophila melanogaster</i> ]	156	58533	1	3.4	6.38	0.06	
<b>78</b>	<b>T11</b>	gij17647245	T-complex chaperonin 5, isoform A [ <i>Drosophila melanogaster</i> ]	146	59697	2	5	5.88	0.11	

Table 2: Heat shock proteins identified in *M. tardigradum* using the tardigrade protein database.

Protein no.	Slice no.	Protein annotation	Protein score	Protein matches (significant unique peptides)	Protein mass	Protein coverage (%)	Protein pI	emPAI	Sum emPAI
<b>Heat shock protein 40 family (J-protein family)</b>									
1	A12	fun1_c4406:232:1473:1 similar to DnaJ homolog subfamily A member 2 (Q2HJ94) Evalue: 5e-93	490	11 (11)	46204	36,1	6,06	1,14	
	T12	fun1_c4406:232:1473:1 similar to DnaJ homolog subfamily A member 2 (Q2HJ94) Evalue: 5e-93	513	13 (11)	46204	40	6,06	1,14	
2	A12	fun1_c552:449:1388:2 similar to DnaJ homolog subfamily B member 11 (Q5RAJ6) Evalue: 3e-97	134	2 (2)	35424	9	5,19	0,2	
	T13, 12	fun1_c552:449:1388:2 similar to DnaJ homolog subfamily B member 11 (Q5RAJ6) Evalue: 3e-97	114	2 (2)	35424	9	5,19	0,2	0,29
3	A12,11	fun1_c5750:1:741:1 similar to DnaJ homolog subfamily A member 1 (Q5E954) Evalue: 1e-62	120	3 (3)	28167	15	5,44	0,4	0,65
	T12,11	fun1_c5750:1:741:1 similar to DnaJ homolog subfamily A member 1 (Q5E954) Evalue: 1e-62	157	3 (3)	28167	15,9	5,44	0,4	0,52
4	A11	fun1_c30342:19:691:2 similar to DnaJ homolog subfamily C member 3 (Q13217) Evalue: 3e-37	114	2 (2)	25478	15,2	7,18	0,28	
5	A14	fun1_c25738:1:653:3 similar to DnaJ homolog subfamily B member 4 (Q9D832) Evalue: 5e-61	100	3 (3)	23966	17,1	9,52	0,48	
	T14	fun1_c25738:1:653:3 similar to DnaJ homolog subfamily B member 4 (Q9D832) Evalue: 5e-61	93	3 (3)	23966	9,7	9,52	0,3	
6	T11	fun1_c7827:361:2115:1 similar to DnaJ homolog subfamily C member 3 (Q5Z113) Evalue: 2e-95	86	3 (3)	66794	4,5	6,18	0,1	
7	T14	fun1_c21251:1:826:3 similar to DnaJ homolog subfamily B member 4 (Q9UDY4) Evalue: 2e-62	77	1	30759	4,7	7,33	0,11	
8	A11,12	fun1_c28015:327:1157:3 similar to DnaJ homolog subfamily A member 1 (Q5NVI9) Evalue: 5e-84	74	2 (2)	31413	7,6	8,44	0,22	0,44
	T12, 11	fun1_c28015:327:1157:3 similar to DnaJ homolog subfamily A member 1 (Q5NVI9) Evalue: 5e-84	98	2 (2)	31413	4	8,44	0,22	0,33
9	T16	fun1_c14868:431:829:2 similar to DnaJ homolog subfamily A member 4 (Q8VW22) Evalue: 4e-05	69	2 (2)	14820	18,9	9,06	0,51	
10	A14	fun1_c26252:325:1461:1 similar to DnaJ homolog subfamily B member 6 (Q6AYU3) Evalue: 1e-38	60	2 (2)	42657	5,8	9,16	0,16	
	T14	fun1_c26252:325:1461:1 similar to DnaJ homolog subfamily B member 6 (Q6AYU3) Evalue: 1e-38	70	2 (2)	42657	5,8	9,16	0,16	
11	A4	fun1_c26005:396:1928:1 similar to DnaJ homolog subfamily C member 13 (O75165) Evalue: 3e-145	43	1	57086	2,2	5,83	0,06	
	T4	fun1_c26005:396:1928:1 similar to DnaJ homolog subfamily C member 13 (O75165) Evalue: 3e-145	77	2 (2)	57086	4,1	5,83	0,12	
12	A8	fun1_c5040:165:1817:3 similar to DnaJ homolog subfamily C member 11 (Q9NVH1) Evalue: 8e-62	26	1	62618	1,1	8,22	0	
13	A10	fun1_c1365:181:1431:1 similar to DnaJ homolog subfamily B member 12 (Q9QYI4) Evalue: 1e-52	25	1	48596	1,4	8,78	0	
	T8	fun1_c1365:181:1431:1 similar to DnaJ homolog subfamily B member 12 (Q9QYI4) Evalue: 1e-52	31	1	48596	1,4	8,78	0,07	
<b>Chaperonin (Hsp60) family</b>									
14	A10, 9-19	fun1_c325:1471:2630:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 5e-151	1541	53 (27)	41076	70,6	4,89	10,03	14,09
	T10, 9-17,19	fun1_c325:1471:2630:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 5e-151	1448	56 (23)	41076	70,9	4,89	7,74	10,48
	A10, 9-12, 14-								
15	19	fun1_c5863:231:1196:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 8e-137	1151	35 (16)	34672	57,8	9,44	4,19	7,78

	<b>T10</b> , 11-19	fun1_c5863:231:1196:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 8e-137	1105	43 (16)	34672	56,8	9,44	5,23	8,23
<b>16</b>	<b>A4</b> , 6	fun1_c10276:276:813:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 1e-54	29	1	19754	4,5	4,86	0,17	0,34
	<b>T4</b> , 6	fun1_c10276:276:813:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 1e-54	28	1	19754	4,5	4,86	0,17	0,34
<b>Heat shock protein 70 family</b>									
<b>17</b>	<b>A9</b> , 8-18	fun1_c3361:227:2224:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	2370	87 (37)	73678	54,9	5,26	7,09	12,48
	<b>T9</b> , 8-18	fun1_c3361:227:2224:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	2222	106 (38)	73678	51	5,26	7,45	9,96
<b>18</b>	<b>A9</b> , 8-17	fun1_c25282:228:2114:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	2216	93 (39)	69515	62,1	5,53	12,27	18,42
	<b>T9</b> , 8-17,19	fun1_c25282:228:2114:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	2223	113 (37)	69515	58,1	5,53	9,54	12,5
<b>19</b>	<b>A9</b> , 8-13, 15-19	fun1_c2228:1:2207:3 similar to Chaperone protein dnaK (A6T4F4) Evalue: 0.0	2100	57 (36)	80766	51	7,75	4,32	6,14
	<b>T9</b> , 8-13,17	fun1_c2228:1:2207:3 similar to Chaperone protein dnaK (A6T4F4) Evalue: 0.0	2084	70 (34)	80766	46,7	7,75	4,99	5,54
<b>20</b>	<b>A8</b> ,9,11-13,16	fun1_c4299:290:2434:2 similar to Heat shock protein 105 kDa (Q5R606) Evalue: 1e-175	1771	44 (29)	81057	53,8	5,3	2,56	2,98
	<b>T8</b> ,11,14	fun1_c4299:290:2434:2 similar to Heat shock protein 105 kDa (Q5R606) Evalue: 1e-175	1916	58 (30)	81057	55,7	5,3	3,7	3,78
<b>21</b>	<b>A9</b> , 8-11	fun1_c2462:685:2595:1 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	1482	40 (22)	70283	39,2	5,28	2,59	3,14
	<b>T9</b> ,10,12	fun1_c2462:685:2595:1 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	1455	49 (22)	70283	41,8	5,28	2,43	2,73
<b>22</b>	<b>A9</b> ,13,16,17	fun1_c166:202:2160:1 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	441	9 (7)	71299	14,1	5,25	0,37	0,69
	<b>T9</b> , 16	fun1_c166:202:2160:1 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	771	19 (13)	71299	23,6	5,25	0,8	0,89
<b>23</b>	<b>A19</b>	fun1_c15295:189:1372:3 similar to Heat shock 70 kDa protein cognate 3 (P29844) Evalue: 0.0	299	4 (4)	44019	13,7	8,59	0,34	
	<b>T19</b>	fun1_c15295:189:1372:3 similar to Heat shock 70 kDa protein cognate 3 (P29844) Evalue: 0.0	198	3 (3)	44019	11,4	8,59	0,24	
<b>24</b>	<b>A9</b>	fun1_c6196:190:2046:1 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	283	9 (5)	68048	10,4	5,19	0,21	
	<b>T9</b>	fun1_c6196:190:2046:1 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	363	15 (7)	68048	19,1	5,19	0,46	
<b>25</b>	<b>A6</b> , 7, 8	fun1_c6150:2022:3834:2 similar to Hypoxia up-regulated protein 1 (Q9Y4L1) Evalue: 1e-34	250	4 (4)	67039	10,3	5,16	0,21	0,36
	<b>T6</b> , 8	fun1_c6150:2022:3834:2 similar to Hypoxia up-regulated protein 1 (Q9Y4L1) Evalue: 1e-34	388	8 (7)	67039	18,7	5,16	0,4	0,45
<b>26</b>	<b>A6</b> , 12	fun1_c28913:70:546:1 similar to Hypoxia up-regulated protein 1 (Q0VA61) Evalue: 3e-34	149	4 (4)	17746	41,1	6,96	1,01	1,2
	<b>T6</b> , 11, 12, 13	fun1_c28913:70:546:1 similar to Hypoxia up-regulated protein 1 (Q0VA61) Evalue: 3e-34	165	5 (3)	17746	29,7	6,96	0,69	1,26
<b>GrpE family</b>									
<b>27</b>	<b>A17</b> , 16	fun1_c2740:265:1029:1 similar to GrpE protein homolog 1, mitochondrial (Q5RA81) Evalue: 1e-47	305	7 (6)	28432	28,7	6,99	0,12	1,06
	<b>T17</b> , 16	fun1_c2740:265:1029:1 similar to GrpE protein homolog 1, mitochondrial (Q5RA81) Evalue: 1e-47	407	9 (7)	28432	35,4	6,99	0,94	1,06
<b>Heat shock protein 90 family</b>									
<b>28</b>	<b>A8</b> , 7-19	fun1_c1107:310:2073:1 similar to Heat shock protein HSP 90-beta (Q5R710) Evalue: 0.0	2209	86 (42)	68508	61,7	5,14	8,47	18,2

	<b>T8</b> , 4,8-19	fun1_c1107:310:2073:1 similar to Heat shock protein HSP 90-beta (Q5R710) Evalue: 0.0	2027	99 (40)	68508	60,2	5,14	6,85	13,52	
<b>29</b>	<b>A8</b> , 5, 7-18	fun1_c542:276:1031:3 similar to Heat shock protein HSP 90-alpha 1 (Q90474) Evalue: 6e-97	1033	40 (17)	28699	67,3	4,85	14,48	22,76	
	<b>T8</b> , 5, 7-16	fun1_c542:276:1031:3 similar to Heat shock protein HSP 90-alpha 1 (Q90474) Evalue: 6e-97	872	36 (15)	28699	66,1	4,85	10,15	13,94	
	<b>A8</b> , 10-13,									
<b>30</b>	16,17	fun1_c2616:123:2231:3 similar to Heat shock protein 83 (P51819) Evalue: 0.0	287	7 (4)	80900	7,1	5,02	0,17	0,65	
	<b>T8</b> ,11,12,16,19	fun1_c2616:123:2231:3 similar to Heat shock protein 83 (P51819) Evalue: 0.0	374	12 (8)	80900	12,3	5,02	0,32	0,6	
<b>31</b>	<b>A5</b>	fun1_c10475:1:443:3 similar to Heat shock protein 83 (O02192) Evalue: 3e-45	43	1	16801	7,5	4,93	0,2		
	<b>T13</b>	fun1_c10475:1:443:3 similar to Heat shock protein 83 (O02192) Evalue: 3e-45	231	9 (5)	16801	32,7	4,93	1,51		
<b>Small heat shock protein (Hsp20) family</b>										
<b>32</b>	<b>A10</b> , 5-19	fun1_lrc24232:253:1327:2 similar to Major egg antigen (P12812) Evalue: 9e-12	1401	84 (19)	37124	76,2	8,5	10,96	42,25	
	<b>T11</b> , 4-19	fun1_lrc24232:253:1327:2 similar to Major egg antigen (P12812) Evalue: 9e-12	1582	106 (20)	37124	78,7	8,5	12,03	41,95	
<b>33</b>	<b>A13</b> , 11-15	fun1_c1023:1:1257:1 similar to Protein lethal(2)essential for life (P82147) Evalue: 1e-11	573	21 (10)	46801	22,5	6,65	1,59	2,99	
	<b>T13</b> , 12-14	fun1_c1023:1:1257:1 similar to Protein lethal(2)essential for life (P82147) Evalue: 1e-11	499	26 (9)	46801	22,2	6,65	1,26	2,48	
<b>34</b>	<b>A17</b>	fun1_c8404:426:1061:1 similar to Major egg antigen (P12812) Evalue: 2e-08	286	6 (6)	23952	43,6	5,98	1,19		
<b>35</b>	<b>A18</b>	fun1_lrc24576:1:662:3 similar to Heat shock protein 67B1 (P05812) Evalue: 4e-13	170	4 (4)	25212	22,3	5,92	0,65		
	<b>T18</b>	fun1_lrc24576:1:662:3 similar to Heat shock protein 67B1 (P05812) Evalue: 4e-13	233	7 (6)	25212	29,1	5,92	1,11		
<b>36</b>	<b>A17</b>	fun1_c2277:509:977:2 similar to Heat shock protein beta-1 (P14602) Evalue: 9e-15	67	2 (2)	17636	13,5	6,97	0,42		
<b>37</b>	<b>A18</b> , 17	fun1_lrc33860:293:919:1 similar to Heat shock protein beta-1 (P14602) Evalue: 8e-15	223	4 (4)	24032	26,4	5,53	0,68	1,1	
	<b>T18</b> , 17	fun1_lrc33860:293:919:1 similar to Heat shock protein beta-1 (P14602) Evalue: 8e-15	282	7 (6)	24032	33,7	5,53	1,18	1,66	
<b>38</b>	<b>A13</b>	fun1_c31148:33:566:3 similar to Major egg antigen (P12812) Evalue: 4e-07	60	1	20608	7,3	8,83	0,16		
	<b>T13</b>	fun1_c31148:33:566:3 similar to Major egg antigen (P12812) Evalue: 4e-07	63	2 (2)	20608	11,9	8,83	0,35		
<b>39</b>	<b>A16</b>	fun1_c22714:224:859:2 similar to Major egg antigen (P12812) Evalue: 1e-07	32	1	24021	10,4	5,98	0,14		
	<b>T17</b>	fun1_c22714:224:859:2 similar to Major egg antigen (P12812) Evalue: 1e-07	242	8 (5)	24021	37	5,98	0,92		
<b>40</b>	<b>A18</b>	fun1_c4695:1:419:3 similar to Major egg antigen (P12812) Evalue: 8e-09	30	1	16231	8,6	5,03	0,21		
	<b>T18</b>	fun1_c4695:1:419:3 similar to Major egg antigen (P12812) Evalue: 8e-09	27	1	16231	8,6	5,03	0,21		
<b>Others</b>										
<b>41</b>	<b>A11</b>	fun1_c4408:395:1926:3 similar to Chaperone activity of bc1 complex-like, mitochondrial (Q5BJQ0) Evalue: 2e-150	215	4 (4)	57543	12,8	8,83	0,25		
	<b>T11</b>	fun1_c4408:395:1926:3 similar to Chaperone activity of bc1 complex-like, mitochondrial (Q5BJQ0) Evalue: 2e-150	424	8 (8)	57543	24,2	8,83	0,56		
<b>42</b>	<b>A13</b>	fun1_c170:449:1869:3 similar to Hsp90 co-chaperone Cdc37 (Q24276) Evalue: 8e-92	140	3 (3)	55402	5,7	5,24	0,19		
	<b>T13</b>	fun1_c170:449:1869:3 similar to Hsp90 co-chaperone Cdc37 (Q24276) Evalue: 8e-92	91	3 (3)	55402	6,1	5,24	0,19		
<b>43</b>	<b>A16</b>	fun1_c2882:1:1009:2 similar to Copper chaperone for superoxide dismutase (O14618) Evalue: 2e-69	85	2 (2)	36390	7,8	6,92	0,19		



	<b>T16</b>	fun1_c2882:1:1009:2 similar to Copper chaperone for superoxide dismutase (O14618) Evalue: 2e-69	31	1	36390	4,2	6,92	0,09
<b>44</b>	<b>A12</b>	fun1_c5060:41:1402:3 similar to Mitochondrial chaperone BCS1 (Q5E9H5) Evalue: 3e-142	77	1	51357	4,2	9,16	0,06
	<b>T12</b>	fun1_c5060:41:1402:3 similar to Mitochondrial chaperone BCS1 (Q5E9H5) Evalue: 3e-142	68	1	51357	4,2	9,16	0,06
<b>45</b>	<b>A13</b>	fun1_c5902:625:1830:1 similar to Activator of 90 kDa heat shock protein ATPase homolog 1 (O95433) Evalue: 1e-82	58	1	46136	2,7	8,76	0,07
	<b>T13</b>	fun1_c5902:625:1830:1 similar to Activator of 90 kDa heat shock protein ATPase homolog 1 (O95433) Evalue: 1e-82	31	1	46136	2,7	8,76	
<b>46</b>	<b>T17</b>	fun1_c10762:4:540:2 similar to Copper chaperone for superoxide dismutase (Q9JK72) Evalue: 2e-40	35	1	19249	4,5	5,75	0,18

Supplementary table 1 and 2 include the peptide sequences of identified proteins listed in table 1 and 2. These two supplementary tables are not included in the thesis because of their extensiveness.

Supplementary table 3. Comparative analysis of Hsp proteins in the active versus the anhydrobiotic (tun) state.

Protein no.	Protein annotation	Sum emPAI [(active)- (tun)]
1	fun1_c4406:232:1473:1 similar to DnaJ homolog subfamily A member 2 (Q2HJ94) Evalue: 5e-93	0
2	fun1_c552:449:1388:2 similar to DnaJ homolog subfamily B member 11 (Q5RAJ6) Evalue: 3e-97	-0.09
3	fun1_c5750:1:741:1 similar to DnaJ homolog subfamily A member 1 (Q5E954) Evalue: 1e-62	0.13
4	fun1_c30342:19:691:2 similar to DnaJ homolog subfamily C member 3 (Q13217) Evalue: 3e-37	0.28
5	fun1_c25738:1:653:3 similar to DnaJ homolog subfamily B member 4 (Q9D832) Evalue: 5e-61	0.18
6	fun1_c7827:361:2115:1 similar to DnaJ homolog subfamily C member 3 (Q5Z113) Evalue: 2e-95	-0.1
7	fun1_c21251:1:826:3 similar to DnaJ homolog subfamily B member 4 (Q9UDY4) Evalue: 2e-62	-0.11
8	fun1_c28015:327:1157:3 similar to DnaJ homolog subfamily A member 1 (Q5NVI9) Evalue: 5e-84	0.11
9	fun1_c14868:431:829:2 similar to DnaJ homolog subfamily A member 4 (Q8WW22) Evalue: 4e-05	-0.51
10	fun1_c26252:325:1461:1 similar to DnaJ homolog subfamily B member 6 (Q6AYU3) Evalue: 1e-38	0
11	fun1_c26005:396:1928:1 similar to DnaJ homolog subfamily C member 13 (O75165) Evalue: 3e-145	-0.06
12	fun1_c5040:165:1817:3 similar to DnaJ homolog subfamily C member 11 (Q9NVH1) Evalue: 8e-62	0
13	fun1_c1365:181:1431:1 similar to DnaJ homolog subfamily B member 12 (Q9QYI4) Evalue: 1e-52	-0.07
14	fun1_c325:1471:2630:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 5e-151	3.61
15	fun1_c5863:231:1196:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 8e-137	-0.45
16	fun1_c10276:276:813:3 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 1e-54	0
17	fun1_c3361:227:2224:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	2.52
18	fun1_c25282:228:2114:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	5.92
19	fun1_c2228:1:2207:3 similar to Chaperone protein dnaK (A6T4F4) Evalue: 0.0	0.6
20	fun1_c4299:290:2434:2 similar to Heat shock protein 105 kDa (Q5R606) Evalue: 1e-175	-0.8
21	fun1_c2462:685:2595:1 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	0.41
22	fun1_c166:202:2160:1 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	-0.2
23	fun1_c15295:189:1372:3 similar to Heat shock 70 kDa protein cognate 3 (P29844) Evalue: 0.0	0.1
24	fun1_c6196:190:2046:1 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	-0.25
25	fun1_c6150:2022:3834:2 similar to Hypoxia up-regulated protein 1 (Q9Y4L1) Evalue: 1e-34	-0.09
26	fun1_c28913:70:546:1 similar to Hypoxia up-regulated protein 1 (Q0VA61) Evalue: 3e-34	-0.06
27	fun1_c2740:265:1029:1 similar to GrpE protein homolog 1, mitochondrial (Q5RA81) Evalue: 1e-47	0
28	fun1_c1107:310:2073:1 similar to Heat shock protein HSP 90-beta (Q5R710) Evalue: 0.0	4.68
29	fun1_c542:276:1031:3 similar to Heat shock protein HSP 90-alpha 1 (Q90474) Evalue: 6e-97	8.82
30	fun1_c2616:123:2231:3 similar to Heat shock protein 83 (P51819) Evalue: 0.0	0.05
31	fun1_c10475:1:443:3 similar to Heat shock protein 83 (O02192) Evalue: 3e-45	-1.31
32	fun1_irc24232:253:1327:2 similar to Major egg antigen (P12812) Evalue: 9e-12	0.3
33	fun1_c1023:1:1257:1 similar to Protein lethal(2)essential for life (P82147) Evalue: 1e-11	0.51
34	fun1_c8404:426:1061:1 similar to Major egg antigen (P12812) Evalue: 2e-08	1.19
35	fun1_irc24576:1:662:3 similar to Heat shock protein 67B1 (P05812) Evalue: 4e-13	-0.46
36	fun1_c2277:509:977:2 similar to Heat shock protein beta-1 (P14602) Evalue: 9e-15	0.42
37	fun1_irc33860:293:919:1 similar to Heat shock protein beta-1 (P14602) Evalue: 8e-15	-0.56
38	fun1_c31148:33:566:3 similar to Major egg antigen (P12812) Evalue: 4e-07	-0.19
39	fun1_c22714:224:859:2 similar to Major egg antigen (P12812) Evalue: 1e-07	-0.78
40	fun1_c4695:1:419:3 similar to Major egg antigen (P12812) Evalue: 8e-09	0
41	fun1_c4408:395:1926:3 similar to Chaperone activity of bc1 complex-like, mitochondrial (Q5BJQ0) Evalue: 2e-150	-0.31
42	fun1_c170:449:1869:3 similar to Hsp90 co-chaperone Cdc37 (Q24276) Evalue: 8e-92	0
43	fun1_c2882:1:1009:2 similar to Copper chaperone for superoxide dismutase (O14618) Evalue: 2e-69	0.1
44	fun1_c5060:41:1402:3 similar to Mitochondrial chaperone BCS1 (Q5E9H5) Evalue: 3e-142	0
45	fun1_c5902:625:1830:1 similar to Activator of 90 kDa heat shock protein ATPase homolog 1 (O95433) Evalue: 1e-82	0.07
46	fun1_c10762:4:540:2 similar to Copper chaperone for superoxide dismutase (Q9JK72) Evalue: 2e-40	-0.18

## 7 Supplementary

### 7.1 Supplementary I

**Identified proteins from preparative 2D gel.** The result of 2D gel analysis have been updated by performing the database search against the recently developed tardigrade protein database based on 454 sequences. Spot number, protein annotation, accession number, total protein score, protein mass, protein pI, number of matched peptides and sequence coverage are listed. A total number of 606 protein spots were analyzed from which 502 (82.8%) protein spots had significant hits.

Spot no.	Protein description	Protein score	Protein mass	Peptide matches (unique peptides)	Protein coverage (%)	Protein pI
A11	contig10543:1:889:2 similar to Filamin-A (Q8BTM8) Evalue: 1e-72	422	31907	7	29,2	8,52
	contig10542:1:699:1 similar to Filamin-C (Q8VHX6) Evalue: 2e-49	88	26529	2	11,6	5,97
A23	contig02694:1:571:2 No Annotation	144	22160	3	18,5	8,74
	contig04802:1:115:695:1 No Annotation	55	21955	1	6,2	6,23
A24	contig02694:1:571:2 No Annotation	66	22160	2	13,8	8,74
A26	contig02694:1:571:2 No Annotation	156	22160	3	19	8,74
A27	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	415	47896	8 (7)	20,6	8,32
A28	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	219	47896	5	14,4	8,32
A29	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	345	47896	9 (7)	20	8,32
A30	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	586	47896	14 (11)	36,9	8,32
A32	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	592	47896	12 (11)	38,3	8,32
	contig24910:1:199:2 No Annotation	32	7556	1	12,3	4,9
A33	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	340	47896	8 (7)	20,6	8,32
A35	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	382	47896	8	27,8	8,32
	contig26438:1:1110:1 similar to Dipeptidyl aminopeptidase-like protein 6 (P46101) Evalue: 2e-43	27	42529	1	3,5	7,53
A40	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	172	47896	4	10,7	8,32
A41	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	216	47896	6 (5)	13	8,32
A42	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	216	47896	6 (5)	13	8,32
A49	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	60	47896	2	4,6	8,32
	contig24910:1:199:2 No Annotation	41	7556	1	12,3	4,9
A50	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	121	47896	4 (3)	9,5	8,32
	contig11006:710:883:2 No Annotation	73	6424	1	26,3	9,52
A59	contig17490:79:1254:1 similar to Uncharacterized serpin-like protein MM_2675 (Q8PTN8) Evalue: 1e-34	276	44072	4	11	8,37
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	139	56002	4	8,1	6,01
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	49	20178	1	9,7	4,84
	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	44	50376	1	1,8	8,51
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	41	35963	1	3,2	9,92
A79	contig23804:63:702:3 similar to Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial (P00348) Evalue: 1e-59	163	22782	3	23	9,35
	contig01757:1:876:1 similar to Adenylyl cyclase-associated protein 1 (Q01518) Evalue: 1e-58	102	32751	2	7,2	8,45
	contig04564:1:305:3 similar to Probable 3-hydroxyacyl-CoA dehydrogenase F54C8.1 (P34439) Evalue: 4e-30	93	11007	2	24	6,1
	contig21807:1:609:2 similar to Chymotrypsin-like protease CTRL-1 (P40313) Evalue: 5e-17	38	22387	1	5	9,15
A81	contig21807:1:609:2 similar to Chymotrypsin-like protease CTRL-1 (P40313) Evalue: 5e-17	140	22387	3	13,9	9,15
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	79	55650	2	4,3	6,86
	contig23401:118:845:1 similar to Voltage-dependent anion-selective channel protein 2 (P81004) Evalue: 2e-41	71	26293	2	11,2	9
	contig19800:1:1133:3 similar to Leukocyte elastase inhibitor A (Q9D154) Evalue: 7e-57	64	41290	1	2,7	9,01
	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	33	50376	1	1,8	8,51
A82	contig00520:1:1212:1 No Annotation	78	46515	1	3	9,43
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	77	35963	2	5,7	9,92
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	41	55650	1	1,9	6,86
A84	contig18366:49:1152:1 similar to Cathepsin K (O35186) Evalue: 3e-31	159	41584	3	10,1	8,48

## Supplementary

	contig17111:429:815:3 No Annotation	58	14772	1	8,6	8,15
	contig19800:1:1133:3 similar to Leukocyte elastase inhibitor A (Q9D154) Evalue: 7e-57	40	41290	1	2,7	9,01
	contig24910:1:199:2 No Annotation	37	7556	2 (1)	12,3	4,9
	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	30	50376	1	1,8	8,51
A85	contig18366:49:1152:1 similar to Cathepsin K (O35186) Evalue: 3e-31	135	41584	3	8,4	8,48
	contig03620:28:725:1 similar to Glucan endo-1,3-beta-glucosidase A1 (P23903) Evalue: 2e-16	91	26688	2	10,3	9,14
	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	60	46522	1	2,6	6,07
	contig19800:1:1133:3 similar to Leukocyte elastase inhibitor A (Q9D154) Evalue: 7e-57	47	41290	1	2,7	9,01
	contig26153:1:1449:2 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	30	53380	1	2,5	6,01
A88	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	76	47896	2	6,3	8,32
A89	contig02499:128:721:2 similar to Prostatic acid phosphatase (P15309) Evalue: 6e-25	213	21711	4	25,8	8,99
	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	86	38936	2	6,7	8,26
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	49	35963	1	3,5	9,92
	contig11075:81:1535:3 similar to Dihydropolyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial (Q9D2G2) Evalue: 6e-109	44	52623	1	2,5	9,53
	contig24910:1:199:2 No Annotation	33	7556	1	12,3	4,9
A90	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	270	35963	6	15,1	9,92
	contig11998:1:493:2 No Annotation	192	18926	3	23,9	8,87
	contig19607:1:514:2 No Annotation	46	19460	1	5,8	9,78
A91	contig19607:1:514:2 No Annotation	173	19460	6 (3)	22,2	9,78
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
A92	contig19607:1:514:2 No Annotation	81	19460	3 (2)	9,4	9,78
A93	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	168	35963	4	12,6	9,92
	contig19607:1:514:2 No Annotation	38	19460	1	4,7	9,78
A94	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	50	35963	1	3,5	9,92
	contig19607:1:514:2 No Annotation	44	19460	1	5,8	9,78
	contig18366:49:1152:1 similar to Cathepsin K (O35186) Evalue: 3e-31	42	41584	1	3	8,48
	contig24910:1:199:2 No Annotation	41	7556	1	12,3	4,9
	contig24912:1:669:1 No Annotation	31	25534	1	5,4	7,91
A95	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	210	35963	5 (4)	12,9	9,92
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
A96	contig20136:95:615:2 similar to Probable methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (Q17M80) Evalue: 2e-57	157	19453	3	19,7	8,98
A97	contig01757:1:876:1 similar to Adenylyl cyclase-associated protein 1 (Q01518) Evalue: 1e-58	63	32751	1	4,8	8,45
A99	contig07413:1:1541:1 similar to 60 kDa heat shock protein, mitochondrial (P63038) Evalue: 0.0	107	55565	2	4,7	5,88
A100	contig26029:1:210:1 No Annotation	61	7775	1	12,9	4,67
A102	contig00037:293:892:2 No Annotation	38	22409	1	8	9,48
A103	contig19541:1:386:2 similar to RNA-binding protein Rsf1 (Q24491) Evalue: 2e-08	117	14047	2	29,7	5,89
A104	contig02499:128:721:2 similar to Prostatic acid phosphatase (P15309) Evalue: 6e-25	146	21711	3	18,2	8,99
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	98	35963	2	6,9	9,92
	contig19541:1:386:2 similar to RNA-binding protein Rsf1 (Q24491) Evalue: 2e-08	45	14047	1	9,4	5,89
	contig27496:1:190:1 similar to Trifunctional enzyme subunit alpha, mitochondrial (P40939) Evalue: 6e-13	44	6970	1	22,2	4,38

## Supplementary

A105	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	164	35963	3	10,4	9,92
	contig19541:1:386:2 similar to RNA-binding protein Rsf1 (Q24491) Evalue: 2e-08	111	14047	3 (2)	29,7	5,89
A106	contig25773:1:614:3 similar to GTP:AMP phosphotransferase mitochondrial (Q9WTP7) Evalue: 7e-42	135	23299	3	18,7	8,52
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	97	35963	2	8,2	9,92
	contig07429:1:196:3 similar to Adenylate kinase isoenzyme 4, mitochondrial (Q9WUS0) Evalue: 3e-07	49	6959	1	17,2	9,9
	contig27496:1:190:1 similar to Trifunctional enzyme subunit alpha, mitochondrial (P40939) Evalue: 6e-13	44	6970	1	22,2	4,38
	contig19541:1:386:2 similar to RNA-binding protein Rsf1 (Q24491) Evalue: 2e-08	42	14047	1	9,4	5,89
A107	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	83	35963	2	8,2	9,92
	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	48	50376	1	2,4	8,51
	contig19607:1:514:2 No Annotation	40	19460	1	4,7	9,78
A108	contig19607:1:514:2 No Annotation	30	19460	1	4,7	9,78
A109	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	49	35963	1	3,5	9,92
A111	contig22522:136:803:1 similar to Prohibitin-2 (Q5XIH7) Evalue: 1e-84	121	24672	2	9,5	9,52
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	93	35963	2	5,7	9,92
A112	contig28250:1:790:2 No Annotation	356	29723	7 (6)	26	9,05
	contig17822:1:826:2 No distinct annotation Best-hit: PDZ and LIM domain protein 3 (Q9PU47) Evalue: 8e-09	220	30151	4	25,5	9,32
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	147	35963	3	16,1	9,92
	contig23401:118:845:1 similar to Voltage-dependent anion-selective channel protein 2 (P81004) Evalue: 2e-41	135	26293	2	11,2	9
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	84	56002	1	3,2	6,01
	contig23830:1096:1332:1 No Annotation	83	8876	1	15,4	8,91
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	57	55650	1	2,3	6,86
	contig02694:1:571:2 No Annotation	55	22160	1	7,9	8,74
A113	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	78	56002	1	3,2	6,01
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	37	35963	1	4,7	9,92
A114	contig28250:1:790:2 No Annotation	60	29723	1	4,2	9,05
	contig04440:420:1643:3 No Annotation	49	46270	1	2,9	6,84
A115	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	53	56002	1	3,2	6,01
A116	contig17641:106:1112:1 similar to Adenosine deaminase (B2T672) Evalue: 7e-81	45	37506	1	2,7	8,3
	contig17822:1:826:2 No distinct annotation Best-hit: PDZ and LIM domain protein 3 (Q9PU47) Evalue: 8e-09	39	30151	1	3,6	9,32
A118	contig23830:1096:1332:1 No Annotation	70	8876	1	15,4	8,91
	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	44	65433	1	2	8,62
A119	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	48	46596	1	3,6	7,71
A121	contig23461:107:1792:2 similar to ATP synthase subunit alpha (AOLDA2) Evalue: 0.0	247	60617	5	10,2	9,23
	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	115	65433	2	4	8,62
	contig24401:1:658:2 similar to Malate dehydrogenase, mitochondrial (Q5NVR2) Evalue: 6e-85	70	23465	1	5,5	8,85
A122	contig09885:332:509:2 similar to Glutathione-requiring prostaglandin D synthase (O60760) Evalue: 2e-12	49	6757	2 (1)	27,1	4,81
	contig24910:1:199:2 No Annotation	36	7556	1	12,3	4,9
A123	contig27733:1:294:1 No Annotation	183	10656	4	54,1	8,07
	contig24910:1:199:2 No Annotation	34	7556	1	12,3	4,9
A126	contig20321:1:864:1 similar to 60S acidic ribosomal protein P0 (Q9U3U0) Evalue: 8e-85	30	30811	1	4,5	8,86
A127	contig18859:1:662:3 No Annotation	505	25323	11 (8)	42,9	8,53
	contig02694:1:571:2 No Annotation	54	22160	1	7,9	8,74

## Supplementary

	contig04802:115:695:1 No Annotation	32	21955	1	6,2	6,23
A128	contig24510:145:671:1 similar to Myophilin (Q24799) Evalue: 4e-31	248	19774	5	31,4	8,31
A129	contig25822:42:1055:3 similar to Probable succinyl-CoA ligase [GDP-forming] subunit alpha, mitochondrial (P53596) Evalue: 2e-110	162	35753	3	14,8	9,2
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	50	56002	1	3,2	6,01
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	49	35963	1	3,5	9,92
A130	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	50	35963	1	3,5	9,92
A131	contig25813:1:416:2 similar to ATP synthase subunit O, mitochondrial (Q2EN81) Evalue: 8e-25	73	15794	1	7,2	9,26
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	62	35963	1	3,5	9,92
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	47	56002	1	2,2	6,01
A132	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	157	35963	4 (3)	10,7	9,92
A133	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	104	56002	2	5,1	6,01
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	50	35963	1	3,5	9,92
	contig13522:1:269:3 No Annotation	39	10952	1	10,1	9,74
A134	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	128	56002	3	6,3	6,01
A136	contig22582:74:622:2 No Annotation	269	20526	6	38,5	8,22
A138	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	68	17856	1	9	6,4
	contig25756:1:500:2 similar to rRNA 2--O-methyltransferase fibrillar (Q9W1V3) Evalue: 4e-69	44	18308	1	6,6	8,54
A143	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	150	35963	3	10,7	9,92
	contig22582:74:622:2 No Annotation	30	20526	1	10,4	8,22
A145	contig24734:209:571:2 No Annotation	34	13132	1	14,9	9,67
A146	contig10105:1:309:3 No Annotation	43	11691	1	14,7	5,63
A147	contig11756:1:1616:1 similar to Advillin (O75366) Evalue: 2e-89	36	60740	1	2,8	9,28
A148	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	363	64906	6 (5)	9,5	5,47
	contig24216:1:620:3 similar to Peptidyl-prolyl cis-trans isomerase (P25007) Evalue: 1e-75	107	22835	2	14,1	7,15
	contig03284:1:276:1 similar to Peptidyl-prolyl cis-trans isomerase, mitochondrial (P30405) Evalue: 1e-36	33	9898	1	9,9	8,63
A149	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	50	47896	1	2,8	8,32
A157	contig02694:1:571:2 No Annotation	33	22160	1	5,8	8,74
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	30	35963	1	3,2	9,92
A158	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	86	35963	3 (2)	3,2	9,92
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
	contig19607:1:514:2 No Annotation	28	19460	1	4,7	9,78
B8	contig25218:1:459:1 similar to Curved DNA-binding protein (Q09184) Evalue: 3e-16	29	16930	1	6,6	5,26
B9	contig07785:1:816:1 No Annotation	37	30617	1	4,8	6,15
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	32	42088	1	3	6,79
B10	contig07785:1:816:1 No Annotation	88	30617	2	8,1	6,15
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	52	42088	1	3	6,79
B18	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	61	56002	2	4,1	6,01
B19	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	39	56002	1	2,2	6,01
B20	contig07785:1:816:1 No Annotation	46	30617	1	4,8	6,15
B21	contig07785:1:816:1 No Annotation	30	30617	1	3,3	6,15

## Supplementary

B22	contig24531:1:590:3 No Annotation	46	22259	1	7,1	4,65
B33	contig10543:1:889:2 similar to Filamin-A (Q8BTM8) Evalue: 1e-72	79	31907	1	4,4	8,52
	contig10542:1:699:1 similar to Filamin-C (Q8VHX6) Evalue: 2e-49	37	26529	1	4,3	5,97
B41	contig23734:1:153:2 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 6e-16	146	5617	3	42	6,02
	contig26373:1:1159:2 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 9e-134	135	42625	3	6,7	7,17
	contig05600:1:307:2 similar to Transketolase (Q5R1W6) Evalue: 2e-12	96	11323	2 (1)	11,8	7,9
	contig24061:1:1415:2 No Annotation	78	54083	1	3	6,5
	contig20201:1:273:1 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 5e-24	36	9668	1	12,2	5,63
B43	contig10543:1:889:2 similar to Filamin-A (Q8BTM8) Evalue: 1e-72	166	31907	2	9,5	8,52
	contig10542:1:699:1 similar to Filamin-C (Q8VHX6) Evalue: 2e-49	44	26529	1	4,3	5,97
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	36	35963	1	3,2	9,92
B47	contig07785:1:816:1 No Annotation	67	30617	2	6,2	6,15
	contig12607:150:967:3 similar to Inactive testicular serine protease 1 (O70169) Evalue: 5e-14	34	29871	1	4	6,85
B48	contig02546:210:1267:3 No Annotation	280	40721	5	18,2	6,72
	contig02694:1:571:2 No Annotation	32	22160	1	5,8	8,74
B49	contig02546:210:1267:3 No Annotation	203	40721	3	10,5	6,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	38	35963	1	3,2	9,92
B54	contig07785:1:816:1 No Annotation	156	30617	5 (3)	13,6	6,15
	contig13522:1:269:3 No Annotation	95	10952	2	23,6	9,74
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	71	42088	2	6	6,79
B60	contig21929:197:901:2 No Annotation	477	23354	7 (6)	39,6	6,38
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	432	17856	8 (6)	50,6	6,4
	contig05847:1:205:2 No Annotation	87	6597	2	65,7	8,42
B61	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	381	17856	8 (7)	51,2	6,4
	contig21929:197:901:2 No Annotation	186	23354	4 (3)	16,2	6,38
	contig05847:1:205:2 No Annotation	71	6597	2	65,7	8,42
B62	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	556	17856	8	51,2	6,4
	contig21929:197:901:2 No Annotation	554	23354	8 (6)	34,5	6,38
	contig05847:1:205:2 No Annotation	36	6597	1	14,9	8,42
B63	contig23192:88:1508:1 similar to Alanine aminotransferase 2 (Q8TD30) Evalue: 3e-172	65	53019	1	2,5	6,2
B64	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	406	17856	7 (6)	50,6	6,4
	contig21929:197:901:2 No Annotation	337	23354	5	27,2	6,38
	contig05847:1:205:2 No Annotation	85	6597	2	65,7	8,42
	contig24910:1:199:2 No Annotation	38	7556	1	12,3	4,9
B65	contig21929:197:901:2 No Annotation	385	23354	6 (5)	34,9	6,38
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	372	17856	7 (6)	34,9	6,4
	contig02546:210:1267:3 No Annotation	89	40721	2	8	6,72
	contig05847:1:205:2 No Annotation	40	6597	1	14,9	8,42
B78	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	109	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	64	20178	1	9,7	4,84
B79	contig03263:1:1127:3 similar to Gelsolin (Q07171) Evalue: 3e-52	138	42916	4	11,2	7,72
	contig21929:197:901:2 No Annotation	94	23354	2	14,9	6,38



## Supplementary

	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	89	17856	2	15,1	6,4
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	56	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	1	9,7	4,84
B80	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	87	35963	2	9,1	9,92
	contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	71	47896	2	4,6	8,32
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	56	56002	1	2,2	6,01
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	36	20178	1	9,7	4,84
B81	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	77	20178	2	22,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	46	35963	1	6	9,92
	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	34	50376	1	1,8	8,51
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
B82	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	263	65433	5	11,3	8,62
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	117	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	98	20178	3 (2)	16,5	4,84
	contig17588:59:1024:2 No Annotation	46	37396	1	2,8	7,55
	contig10542:1:699:1 similar to Filamin-C (Q8VHX6) Evalue: 2e-49	30	26529	1	4,3	5,97
B83	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	133	20178	5 (3)	29,5	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	106	35963	2	9,1	9,92
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	104	17856	2	16,9	6,4
	contig03062:1:281:2 No Annotation	56	11033	1	11,8	9,23
	contig24910:1:199:2 No Annotation	33	7556	1	12,3	4,9
B84	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	227	17856	4	29,5	6,4
	contig00630:298:996:1 No Annotation	78	25952	1	6	5,26
	contig05847:1:205:2 No Annotation	68	6597	2	65,7	8,42
	contig21929:197:901:2 No Annotation	66	23354	1	6	6,38
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	1	9,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	36	35963	1	6	9,92
	contig03263:1:1127:3 similar to Gelsolin (Q07171) Evalue: 3e-52	34	42916	1	2,9	7,72
B86	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	146	17856	3	24,1	6,4
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	96	56002	2	4,7	6,01
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	74	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	49	20178	2 (1)	9,7	4,84
	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	44	50376	1	2,4	8,51
B87	contig26482:1:1206:1 similar to Medium-chain specific acyl-CoA dehydrogenase, mitochondrial (P41367) Evalue: 279 6e-150	279	44533	4	14,7	6,79
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	53	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	37	20178	1	9,7	4,84
B88	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	200	42088	5 (4)	12,2	6,79
B89	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	176	42088	3	9,8	6,79
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	57	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	49	20178	1	9,7	4,84
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9

## Supplementary

B90	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	118	17856	2	22,3	6,4
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	104	35963	3 (2)	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	2 (1)	9,7	4,84
	contig24910:1:199:2 No Annotation	32	7556	1	12,3	4,9
B91	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	112	17856	2	16,3	6,4
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	108	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	64	20178	2 (1)	9,7	4,84
B92	contig25483:1682:2388:3 similar to Isocitrate dehydrogenase [NADP] cytoplasmic (Q9XSG3) Evalue: 8e-107	250	26858	5	23,5	6,26
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	43	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	36	20178	1	9,7	4,84
B93	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	222	17856	5	34,3	6,4
	contig21929:197:901:2 No Annotation	86	23354	2	10,2	6,38
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	54	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	49	20178	2 (1)	9,7	4,84
	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	30	65433	1	1,9	8,62
B94	contig21929:197:901:2 No Annotation	189	23354	2	12,8	6,38
	contig03062:1:281:2 No Annotation	71	11033	1	12,9	9,23
B95	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	183	42088	4 (3)	9,8	6,79
	contig26482:1:1206:1 similar to Medium-chain specific acyl-CoA dehydrogenase, mitochondrial (P41367) Evalue: 135 6e-150	135	44533	2	6,2	6,79
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	64	20178	1	9,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	57	35963	1	3,2	9,92
B96	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	109	42088	2	7,9	6,79
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	83	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	36	20178	2 (1)	9,7	4,84
B97	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	49	20178	1	9,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	44	35963	1	3,2	9,92
B98	contig26710:1:1127:3 similar to Obg-like ATPase 1 (Q7ZWM6) Evalue: 1e-155	172	42366	3	11,8	6,08
	contig24910:1:199:2 No Annotation	34	7556	1	12,3	4,9
B99	contig04802:115:695:1 No Annotation	116	21955	2	11,4	6,23
	contig02694:1:571:2 No Annotation	47	22160	1	4,8	8,74
B101	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	112	38936	1	3,8	8,26
	contig25609:1:118:2 No Annotation	42	4434	1	23,7	4,87
	contig01051:8:172:2 similar to Trifunctional enzyme subunit alpha, mitochondrial (Q64428) Evalue: 1e-12	39	6378	1	20,4	9,58
B102	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	239	38936	5	27	8,26
	contig02694:1:571:2 No Annotation	168	22160	3	13,2	8,74
	contig04802:115:695:1 No Annotation	153	21955	4 (3)	19,2	6,23
	contig25609:1:118:2 No Annotation	148	4434	3	94,7	4,87
B103	contig04802:115:695:1 No Annotation	33	21955	1	5,2	6,23
B104	contig00405:208:1710:1 similar to 4-aminobutyrate aminotransferase, mitochondrial (P50554) Evalue: 2e-159	64	56682	1	1,8	8,46
B105	contig04802:115:695:1 No Annotation	152	21955	4 (2)	14	6,23
	contig02694:1:571:2 No Annotation	78	22160	2 (1)	7,9	8,74

## Supplementary

	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	49	17856	1	10,2	6,4
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
B107	contig04802:115:695:1 No Annotation	27	21955	1	5,2	6,23
B108	contig02694:1:571:2 No Annotation	91	22160	1	7,9	8,74
	contig26373:1:1159:2 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 9e-134	85	42625	2	4,1	7,17
	contig04802:115:695:1 No Annotation	68	21955	1	6,2	6,23
	contig20201:1:273:1 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 5e-24	59	9668	1	12,2	5,63
B109	contig24401:1:658:2 similar to Malate dehydrogenase, mitochondrial (Q5NVR2) Evalue: 6e-85	152	23465	2	12,8	8,85
B110	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	277	56002	7 (6)	14	6,01
	contig04802:115:695:1 No Annotation	76	21955	1	6,2	6,23
B111	contig02694:1:571:2 No Annotation	66	22160	1	7,9	8,74
	contig04802:115:695:1 No Annotation	58	21955	1	6,2	6,23
B112	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	143	17856	4	26,5	6,4
	contig06624:111:986:3 No Annotation	134	33305	2	9,6	8,17
B133	contig23004:1:974:3 similar to Peptide-N(4)-(N-acetyl-beta-glucosaminy)l asparagine amidase (Q28YQ7) Evalue: 5e-26	254	37038	6 (5)	14,2	9,23
	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	211	41570	4	9,9	6,6
	contig07583:24:941:3 similar to Lipoma-preferred partner homolog (Q5F464) Evalue: 8e-70	31	36195	1	3,3	8,43
B138	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	258	56002	6	12,6	6,01
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	134	17856	3	24,1	6,4
B140	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	221	56002	6 (5)	12,8	6,01
	contig01745:31:1008:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 1e-21	100	36669	2	7,7	5,89
B141	contig09502:237:2105:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	69	68965	1	2,3	5,41
B142	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	533	46522	11 (8)	23	6,07
B143	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	178	17856	3	19,3	6,4
	contig26098:1:627:1 similar to Glutathione S-transferase omega-1 (P78417) Evalue: 6e-39	69	24260	2	11	8,58
	contig05847:1:205:2 No Annotation	28	6597	1	14,9	8,42
B144	contig26153:1:1449:2 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	259	53380	5	12	6,01
	contig09502:237:2105:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	256	68965	5	10,1	5,41
	contig04802:115:695:1 No Annotation	63	21955	1	6,2	6,23
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	37	17856	1	7,2	6,4
B145	contig23157:49:1047:1 similar to Protein NipSnap (Q9VXK0) Evalue: 5e-68	96	38414	2	6,3	9,18
B146	contig26153:1:1449:2 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	526	53380	10 (8)	19,3	6,01
	contig09502:237:2105:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	338	68965	7 (5)	10,1	5,41
	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	179	46522	4 (3)	6,6	6,07
B147	contig22215:21:483:3 similar to 3-hydroxyacyl-CoA dehydrogenase type-2 (O18404) Evalue: 1e-23	44	16933	2 (1)	11	5,42
	contig27778:1:335:3 similar to 3-hydroxyacyl-CoA dehydrogenase type-2 (O08756) Evalue: 5e-29	39	11844	1	10	9,19
B148	contig20526:1:608:3 similar to Malate dehydrogenase, cytoplasmic (O48905) Evalue: 3e-39	144	22687	3	15,4	6,13
	contig02052:156:636:3 similar to Malate dehydrogenase, cytoplasmic (P40925) Evalue: 5e-56	127	17605	2	21,9	8,52
	contig23614:1:531:1 No Annotation	28	18399	1	6,2	8,93
B149	contig26098:1:627:1 similar to Glutathione S-transferase omega-1 (P78417) Evalue: 6e-39	41	24260	1	4,8	8,58

## Supplementary

B150	contig24984:2765:3460:3 similar to FMN-dependent NADPH-azoreductase (O07529) Evalue: 2e-08	137	26030	4	16	9,19
	contig03403:1:1416:3 similar to Dihydropyridine reductase component of pyruvate dehydrogenase complex, mitochondrial (Q8BMF4) Evalue: 6e-108	101	50746	2	4,2	8,63
B151	contig24984:2765:3460:3 similar to FMN-dependent NADPH-azoreductase (O07529) Evalue: 2e-08	55	26030	1	4,3	9,19
B152	contig18259:2282:2926:2 similar to Glutathione-requiring prostaglandin D synthase (O60760) Evalue: 8e-31	110	25248	3 (2)	10,7	8,68
	contig23461:107:1792:2 similar to ATP synthase subunit alpha (A0LDA2) Evalue: 0.0	92	60617	2	3,6	9,23
	contig18624:208:876:1 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-34	66	25396	2	8,6	7,85
B153	contig28284:1:272:3 similar to Glutathione S-transferase (P30116) Evalue: 2e-10	50	10701	1	13,5	4,97
	contig22559:1:111:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-05	43	4476	1	24,3	4,94
	contig27737:1:155:3 No Annotation	39	6370	2 (1)	19,6	4,52
B154	contig22559:1:111:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-05	46	4476	1	24,3	4,94
B155	contig22391:1:1506:1 similar to Acyl-CoA synthetase short-chain family member 3, mitochondrial (Q14DH7) Evalue: 2e-146	67	56504	1	3	7,45
	contig24910:1:199:2 No Annotation	36	7556	1	12,3	4,9
B156	contig17510:1:111:1 No Annotation	55	4280	1	40,5	4,21
	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	50	65433	1	2	8,62
B157	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	37	41570	1	2,5	6,6
B158	contig28200:1:335:1 similar to Glutathione S-transferase Mu 1 (Q9N0V4) Evalue: 4e-19	30	13006	1	8,1	5,82
B159	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	171	41570	5	15,2	6,6
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	77	17856	1	9	6,4
	contig26339:1:396:1 No Annotation	51	15458	1	8,4	7,93
B160	contig01724:145:861:1 similar to Dihydropteridine reductase (P11348) Evalue: 2e-62	85	25377	2	11,3	6,97
B161	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	52	65433	1	1,9	8,62
B162	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	55	41570	1	3	6,6
B163	contig24083:75:995:3 similar to Triosephosphate isomerase (B0BM40) Evalue: 1e-90	95	34276	2	6,9	7,6
B164	contig23461:107:1792:2 similar to ATP synthase subunit alpha (A0LDA2) Evalue: 0.0	99	60617	3	6,4	9,23
B165	contig19542:93:722:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 1e-31	73	23872	1	4,8	8,59
	contig24910:1:199:2 No Annotation	37	7556	1	12,3	4,9
B166	contig28200:1:335:1 similar to Glutathione S-transferase Mu 1 (Q9N0V4) Evalue: 4e-19	71	13006	2	10,8	5,82
	contig22559:1:111:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-05	43	4476	1	24,3	4,94
B167	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	325	41570	8 (7)	22,9	6,6
	contig22559:1:111:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-05	41	4476	1	24,3	4,94
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
B168	contig00490:282:923:3 similar to Cytochrome b-c1 complex subunit Rieske, mitochondrial (Q69BJ7) Evalue: 3e-62	114	23942	2	9,9	8,85
B169	contig19542:93:722:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 1e-31	48	23872	1	4,3	8,59
B173	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	402	46522	13 (9)	25,8	6,07
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	80	17856	1	9	6,4
	contig24984:2765:3460:3 similar to FMN-dependent NADPH-azoreductase (O07529) Evalue: 2e-08	71	26030	2	11,3	9,19
	contig24910:1:199:2 No Annotation	36	7556	1	12,3	4,9
B174	contig24078:1:648:1 similar to Guanine nucleotide-binding protein subunit beta-2-like 1 (Q93134) Evalue: 1e-68	130	24733	3	16,7	8,44
B176	contig24910:1:199:2 No Annotation	33	7556	1	12,3	4,9
B178	contig17641:106:1112:1 similar to Adenosine deaminase (B2T672) Evalue: 7e-81	55	37506	1	2,7	8,3

## Supplementary

B181	contig23852:3162:3608:1 similar to Troponin I (P36188) Evalue: 2e-23	129	17536	3	25,7	7,93
B182	contig19588:68:937:2 similar to ATP synthase subunit gamma, mitochondrial (P05631) Evalue: 1e-97	136	32022	3 (2)	9,3	9,2
B183	contig18110:116:595:2 similar to Peroxiredoxin-5, mitochondrial (Q9GLW9) Evalue: 2e-44	73	17501	1	10,7	6,82
B184	contig10105:1:309:3 No Annotation	102	11691	2	26,5	5,63
B185	contig10105:1:309:3 No Annotation	62	11691	1	11,8	5,63
	contig24910:1:199:2 No Annotation	36	7556	1	12,3	4,9
B187	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
B188	contig24910:1:199:2 No Annotation	41	7556	1	12,3	4,9
	contig25218:1:459:1 similar to Curved DNA-binding protein (Q09184) Evalue: 3e-16	33	16930	1	6,6	5,26
B189	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
B190	contig24227:1:649:2 similar to Translocon-associated protein subunit delta (Q62186) Evalue: 6e-14	339	23584	7	33	8,8
	contig24910:1:199:2 No Annotation	41	7556	1	12,3	4,9
B191	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	96	35963	2	9,1	9,92
	contig24227:1:649:2 similar to Translocon-associated protein subunit delta (Q62186) Evalue: 6e-14	35	23584	1	5,1	8,8
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
B192	contig17641:106:1112:1 similar to Adenosine deaminase (B2T672) Evalue: 7e-81	166	37506	3	12,5	8,3
	contig00614:1:226:2 No Annotation	48	8491	1	10,8	9,78
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
B193	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	52	35963	1	3,2	9,92
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
B194	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
B195	contig24910:1:199:2 No Annotation	41	7556	1	12,3	4,9
B196	contig24078:1:648:1 similar to Guanine nucleotide-binding protein subunit beta-2-like 1 (Q93134) Evalue: 1e-68	101	24733	2	8,8	8,44
B198	contig18504:154:1359:1 similar to Calreticulin (Q4R6K8) Evalue: 4e-127	95	46442	2	5,2	4,66
C2	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	150	55650	3	7,2	6,86
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	117	33965	3	9,9	6,71
	contig24531:1:590:3 No Annotation	105	22259	2	13,3	4,65
C4	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	28	56002	1	2,8	6,01
C5	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	275	55650	5	11	6,86
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	231	33965	5	16,8	6,71
	contig24531:1:590:3 No Annotation	162	22259	4 (3)	19,4	4,65
C6	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	295	55650	6	13,4	6,86
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	230	33965	5	16,8	6,71
	contig24531:1:590:3 No Annotation	150	22259	4 (3)	19,4	4,65
C7	contig24531:1:590:3 No Annotation	88	22259	2	13,3	4,65
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	57	33965	2	6,3	6,71
C9	contig24531:1:590:3 No Annotation	204	22259	4	23,5	4,65
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	165	55650	3	7,4	6,86
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	126	33965	3	9,9	6,71
C16	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	552	56002	13 (12)	28,8	6,01
	contig18438:8:349:2 No Annotation	93	12958	2	23	8,58
C17	contig01298:1:2065:3 similar to Catalase HPII (P21179) Evalue: 0.0	241	76798	6 (5)	10,6	5,98

## Supplementary

C18	contig01298:1:2065:3 similar to Catalase HPII (P21179) Evalue: 0.0	337	76798	8 (7)	12,8	5,98
C19	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	53	42088	1	3	6,79
C20	contig24531:1:590:3 No Annotation	28	22259	1	6,1	4,65
C21	contig01298:1:2065:3 similar to Catalase HPII (P21179) Evalue: 0.0	40	76798	1	1,6	5,98
C22	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	133	42088	3	10,3	6,79
	contig07785:1:816:1 No Annotation	74	30617	1	4,8	6,15
	contig18537:1:1312:2 No Annotation	45	49479	1	2,1	5,84
C22	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	271	56002	6 (5)	11	6,01
	contig18438:8:349:2 No Annotation	42	12958	1	15	8,58
C23	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	221	55650	5	12	6,86
	contig24531:1:590:3 No Annotation	192	22259	5 (4)	23,5	4,65
C25	contig24531:1:590:3 No Annotation	31	22259	1	7,1	4,65
C26	contig24531:1:590:3 No Annotation	41	22259	1	7,1	4,65
C27	contig18438:8:349:2 No Annotation	52	12958	1	9,7	8,58
C28	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	157	56002	5 (4)	9,1	6,01
	contig18438:8:349:2 No Annotation	39	12958	1	9,7	8,58
C30	contig24531:1:590:3 No Annotation	86	22259	2	13,3	4,65
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	59	55650	1	2,3	6,86
C31	contig07785:1:816:1 No Annotation	42	30617	1	4,8	6,15
	contig18537:1:1312:2 No Annotation	33	49479	1	2,1	5,84
C32	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	126	33965	3	9,9	6,71
	contig24531:1:590:3 No Annotation	126	22259	3	17,3	4,65
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	63	55650	2	3,9	6,86
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	41	56002	1	2,2	6,01
C33	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	606	56002	15 (12)	30	6,01
	contig18438:8:349:2 No Annotation	127	12958	3	33,6	8,58
C34	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	86	33965	2	6,3	6,71
	contig24531:1:590:3 No Annotation	59	22259	1	7,1	4,65
C35	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	53	56002	1	2,2	6,01
C36	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	53	42088	1	3	6,79
C37	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	36	56002	1	1,6	6,01
C38	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	64	55650	1	2,3	6,86
C41	contig14499:1:464:3 similar to Alpha-2-macroglobulin-like protein 1 (A8K2U0) Evalue: 9e-05	246	17495	6 (4)	30,1	5,54
	contig25155:1:869:3 No Annotation	27	32421	1	2,4	5,36
C47	contig23758:1:2385:1 similar to Aconitate hydratase, mitochondrial (P20004) Evalue: 0.0	186	86924	5	8,1	8,09
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	40	42088	1	4,9	6,79
C50	contig24531:1:590:3 No Annotation	88	22259	2	13,3	4,65
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	43	55650	1	1,9	6,86
C51	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	58	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	1	9,7	4,84
	contig13035:1:170:1 No Annotation	42	6468	1	14,3	5,25
	contig24910:1:199:2 No Annotation	37	7556	1	12,3	4,9

## Supplementary

	contig08625:1:110:2 No Annotation	35	4172	1	22,2	9,05
C52	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	455	22292	9	52,5	5,26
	contig18449:1:1229:3 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 2e-87	403	44803	8	21,8	8,14
C59	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	271	56002	7	15,8	6,01
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	56	17856	1	9	6,4
	contig01620:1:1397:3 similar to Gamma-glutamyltranspeptidase 1 (P07314) Evalue: 4e-88	50	51178	1	2,8	8,59
C60	contig00798:1:1071:1 similar to Alpha-aminoacidic semialdehyde dehydrogenase (P49419) Evalue: 4e-131	191	39000	4	13,7	5,36
	contig01620:1:1397:3 similar to Gamma-glutamyltranspeptidase 1 (P07314) Evalue: 4e-88	68	51178	2	4,5	8,59
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	59	56002	2	6,7	6,01
	contig18438:8:349:2 No Annotation	42	12958	1	9,7	8,58
C62	contig00023:136:1154:1 No Annotation	199	37758	4	14,7	5,95
C65	contig01298:1:2065:3 similar to Catalase HP11 (P21179) Evalue: 0.0	150	76798	4	6,7	5,98
	contig17939:1:834:1 similar to Pyruvate kinase isozymes M1/M2 (P14618) Evalue: 7e-94	31	31042	1	4	8,29
	contig05711:251:901:2 similar to Pyruvate kinase isozyme M1 (P11979) Evalue: 7e-73	28	23916	1	6	5,05
C71	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	118	20178	4 (2)	16,5	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	107	35963	3 (2)	9,1	9,92
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	85	33965	2	6,9	6,71
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	36	17856	1	13,3	6,4
C78	contig05878:180:1217:3 No Annotation	38	39081	1	3,8	5,72
C83	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	225	42088	5 (4)	12,2	6,79
	contig08235:860:1596:2 No Annotation	55	27856	1	4,1	5,65
	contig17807:1:460:2 similar to Glutamine synthetase (P51121) Evalue: 9e-67	41	16934	1	8,6	6,71
C85	contig24956:1:847:3 similar to Phosphoglycerate kinase (P51903) Evalue: 2e-108	121	30960	2	8,9	5,85
C86	contig19694:48:1100:3 similar to Sulfotransferase 1C2 (Q9D939) Evalue: 2e-44	196	41321	4	7,4	6,39
	contig26710:1:1127:3 similar to Obg-like ATPase 1 (Q7ZWM6) Evalue: 1e-155	137	42366	3	11,2	6,08
C87	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	166	42088	3	9,8	6,79
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	110	33965	3	9,6	6,71
	contig09521:127:1221:1 similar to GDP-mannose 4,6 dehydratase (Q8K0C9) Evalue: 8e-158	60	41533	1	3,3	6,16
C91	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	239	41570	6 (5)	13,5	6,6
C95	contig24463:296:1435:2 similar to Glucose and ribitol dehydrogenase (Q5KTS5) Evalue: 1e-80	415	41808	8 (7)	20,8	8,49
C98	contig24199:214:1227:1 similar to Uncharacterized protein ZK1073.1 (O02485) Evalue: 2e-70	81	38027	2	7,4	6,33
	contig01972:1:390:1 similar to Protein lethal(2)essential for life (P82147) Evalue: 1e-12	44	14859	1	11,5	5,57
	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	34	38936	1	2,9	8,26
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	31	35963	1	3,2	9,92
	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	30	41570	1	2,5	6,6
C100	contig05711:251:901:2 similar to Pyruvate kinase isozyme M1 (P11979) Evalue: 7e-73	232	23916	5	28,6	5,05
	contig17939:1:834:1 similar to Pyruvate kinase isozymes M1/M2 (P14618) Evalue: 7e-94	46	31042	1	4	8,29
	contig15875:1:392:3 similar to Pyruvate kinase muscle isozyme (Q92122) Evalue: 6e-34	38	14587	1	10	5,82
C101	contig07785:1:816:1 No Annotation	152	30617	5 (3)	14,7	6,15
C102	contig07785:1:816:1 No Annotation	107	30617	2	8,1	6,15
C103	contig08799:1:1630:2 similar to Citrate synthase, mitochondrial (Q28DK1) Evalue: 0.0	58	60830	1	1,8	8,53
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	27	42088	1	1,9	6,79

## Supplementary

C104	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	33	41570	1	2,5	6,6
C105	contig08799:1:1630:2 similar to Citrate synthase, mitochondrial (Q28DK1) Evalue: 0.0	50	60830	1	2,4	8,53
C106	contig24527:81:472:3 similar to Fructose-bisphosphate aldolase C (P53448) Evalue: 7e-46	120	14046	2	16,9	5,84
	contig25519:1:245:2 similar to Fructose-bisphosphate aldolase, muscle type (P53445) Evalue: 3e-36	62	9232	1	19,8	5,24
	contig05470:1:464:3 similar to Fructose-bisphosphate aldolase A (P05065) Evalue: 6e-54	37	16379	1	5,2	9,39
C109	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	109	46596	3	7,2	7,71
C110	contig24527:81:472:3 similar to Fructose-bisphosphate aldolase C (P53448) Evalue: 7e-46	394	14046	8 (7)	56,9	5,84
	contig00766:103:1068:1 similar to Quinone oxidoreductase (P47199) Evalue: 6e-76	169	34835	3	13,4	6,31
	contig25519:1:245:2 similar to Fructose-bisphosphate aldolase, muscle type (P53445) Evalue: 3e-36	62	9232	1	19,8	5,24
	contig05470:1:464:3 similar to Fructose-bisphosphate aldolase A (P05065) Evalue: 6e-54	40	16379	1	5,2	9,39
	contig12426:117:740:3 No Annotation	31	23403	1	3,9	9,13
C111	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	821	46596	20 (17)	44	7,71
C112	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	60	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	2 (1)	9,7	4,84
C113	contig23241:1:1690:2 No distinct annotation Best-hit: Four and a half LIM domains protein 2 (Q14192) Evalue: 1e-88	159	66165	5 (4)	7,8	7,23
	contig10939:1:797:2 No Annotation	40	29117	1	6,8	6,96
C114	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	1	9,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	49	35963	1	3,2	9,92
C115	contig10939:1:797:2 No Annotation	246	29117	5 (4)	20,4	6,96
	contig22415:1:289:3 similar to Tryptase gamma (Q9NRR2) Evalue: 2e-10	63	10316	2	27,4	6,75
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
	contig18321:1:789:3 similar to Chymotrypsin-like elastase family member 2A (P08419) Evalue: 3e-23	28	29034	1	3,4	5,56
C116	contig10939:1:797:2 No Annotation	147	29117	3	14	6,96
C117	contig08235:860:1596:2 No Annotation	152	27856	3	15,9	5,65
	contig10939:1:797:2 No Annotation	151	29117	4 (3)	16,2	6,96
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	64	42088	1	3	6,79
	contig21929:197:901:2 No Annotation	39	23354	1	6	6,38
	contig08235:1:820:2 No Annotation	38	30752	1	5,5	8,66
	contig24910:1:199:2 No Annotation	33	7556	1	12,3	4,9
	contig04234:1:1412:1 similar to Plasma kallikrein (P26262) Evalue: 5e-24	30	51095	1	3,4	8,39
C118	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	61	56002	1	2,2	6,01
C119	contig20530:1:997:2 similar to Electron transfer flavoprotein subunit alpha, mitochondrial (Q8HXY0) Evalue: 3e-107	68	34752	1	4,8	8,64
C121	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	64	20178	1	9,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	61	35963	1	3,2	9,92
C122	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	79	42088	2	5,4	6,79
C123	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	111	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	2 (1)	9,7	4,84
C124	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	148	42088	4 (3)	15,5	6,79
	contig24910:1:199:2 No Annotation	34	7556	1	12,3	4,9
C126	contig01844:1:445:1 No Annotation	167	15778	3 (2)	20,3	6,05
	contig06946:143:937:2 similar to Proteasome subunit alpha type-4 (P25789) Evalue: 1e-97	84	29694	2	6,8	6,37



## Supplementary

	contig23845:1:545:3 similar to Coiled-coil domain-containing protein 25 (Q78PG9) Evalue: 5e-37	50	21197	1	6,7	6,54
	contig08317:72:2177:3 similar to GMP synthase [glutamine-hydrolyzing] (Q4V7C6) Evalue: 0.0	49	79072	1	1,7	6,08
	contig26638:1:767:3 similar to Probable actin-related protein 2/3 complex subunit 2 (Q9VIM5) Evalue: 1e-105	38	29666	1	3,9	8,99
C128	contig20321:1:864:1 similar to 60S acidic ribosomal protein P0 (Q9U3U0) Evalue: 8e-85	755	30811	12 (10)	43,2	8,86
	contig18941:155:934:2 No Annotation	48	27813	1	4,2	5,83
	contig24910:1:199:2 No Annotation	33	7556	1	12,3	4,9
C129	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	95	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	94	20178	2	16,5	4,84
	contig10844:1:307:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 7e-25	67	10508	1	10,9	6,02
	contig10939:1:797:2 No Annotation	40	29117	1	6,8	6,96
C131	contig12271:1:402:2 similar to Heat shock cognate 71 kDa protein (P19120) Evalue: 2e-47	120	14834	2	18	5,88
	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	94	46522	2	4,7	6,07
	contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	42	42823	1	3,9	5,27
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
C132	contig24578:1:638:1 similar to Peroxiredoxin-4 (O08807) Evalue: 8e-51	173	23970	3	11,8	6,72
	contig23916:1:215:3 similar to Peroxiredoxin-2 (Q8K3U7) Evalue: 7e-30	51	7931	2 (1)	15,7	5,69
	contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	30	42823	1	3,9	5,27
C133	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	193	46522	4	11,1	6,07
C134	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	121	65433	2	4,6	8,62
	contig21632:1:563:1 similar to Superoxide dismutase [Mn], mitochondrial (P09671) Evalue: 3e-44	92	20772	3 (2)	10,2	5,97
C137	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	189	41570	5	13,5	6,6
C139	contig19529:448:736:1 similar to ATP synthase subunit d, mitochondrial (Q24251) Evalue: 6e-25	81	11005	2	21,9	8,89
C141	contig08235:860:1596:2 No Annotation	416	27856	9 (7)	36,7	5,65
	contig01555:212:841:2 similar to S-crystallin 4 (P27012) Evalue: 3e-25	84	24208	2	12,9	6,43
	contig08235:1:820:2 No Annotation	44	30752	1	5,5	8,66
C142	contig08235:860:1596:2 No Annotation	92	27856	2	9,4	5,65
	contig27809:822:1400:3 No Annotation	61	21769	1	5,7	6,81
	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	53	65433	1	1,9	8,62
C143	contig24083:75:995:3 similar to Triosephosphate isomerase (B0BM40) Evalue: 1e-90	350	34276	7 (6)	25,2	7,6
	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	289	46596	5	15,9	7,71
C144	contig19308:1:1054:3 similar to Serpin B3 (P29508) Evalue: 2e-45	250	39317	5 (4)	16	5,37
C145	contig08235:860:1596:2 No Annotation	139	27856	3	17,1	5,65
	contig24044:157:536:1 similar to Peroxiredoxin-2 (Q2PFZ3) Evalue: 2e-33	76	14240	2	21,4	5,61
	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	66	46596	1	3,4	7,71
	contig21646:1:212:3 similar to Peroxiredoxin-2 (Q8K3U7) Evalue: 7e-29	54	7722	1	15,9	6,07
	contig13693:996:1729:3 No Annotation	27	27448	1	2,9	8,71
C149	contig24248:1:1048:2 similar to Isocitrate dehydrogenase [NAD] subunit gamma, mitochondrial (P70404) Evalue: 9e-113	55	38992	1	4,3	6,64
C150	contig17128:1:666:1 similar to Superoxide dismutase [Cu-Zn] (Q8HXQ3) Evalue: 5e-52	66	23719	1	5,4	6,16
C155	contig07268:293:706:2 similar to Superoxide dismutase [Cu-Zn] (Q8HXQ2) Evalue: 2e-07	99	15341	2	10,9	9,33
	contig02159:1:486:1 similar to Death-associated protein 1 (P51397) Evalue: 5e-05	56	17926	1	7,5	8,74
	contig06938:1:111:1 similar to Superoxide dismutase [Cu-Zn] (O46412) Evalue: 2e-08	48	4060	2 (1)	51,4	5,97

## Supplementary

C156	contig28252:1:509:3 No Annotation	273	18780	5	25,6	8,49
D2	contig08625:1:110:2 No Annotation	188	4172	4 (3)	69,4	9,05
	contig26443:1:303:1 No Annotation	120	11655	2	23,8	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	93	35963	2	9,1	9,92
	contig13035:1:170:1 No Annotation	42	6468	1	14,3	5,25
D3	contig08625:1:110:2 No Annotation	145	4172	3	69,4	9,05
	contig26443:1:303:1 No Annotation	117	11655	2	23,8	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	94	35963	2	9,1	9,92
	contig03062:1:281:2 No Annotation	94	11033	2	24,7	9,23
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	66	20178	2 (1)	9,7	4,84
D4	contig13035:1:170:1 No Annotation	43	6468	1	14,3	5,25
	contig08625:1:110:2 No Annotation	155	4172	3	69,4	9,05
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	104	35963	2	9,1	9,92
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	49	24857	1	5,6	4,86
D5	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	37	20178	1	9,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	60	35963	1	3,2	9,92
	contig13035:1:170:1 No Annotation	51	6468	1	14,3	5,25
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	41	20178	2 (1)	9,7	4,84
D8	contig08625:1:110:2 No Annotation	38	4172	1	22,2	9,05
	contig08625:1:110:2 No Annotation	208	4172	4 (3)	72,2	9,05
	contig26443:1:303:1 No Annotation	159	11655	3	42,6	4,72
	contig03062:1:281:2 No Annotation	128	11033	2	24,7	9,23
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	105	35963	2	9,1	9,92
	contig13035:1:170:1 No Annotation	95	6468	2	57,1	5,25
D10	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	65	20178	2 (1)	9,7	4,84
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	40	24857	1	10,2	4,86
	contig08625:1:110:2 No Annotation	170	4172	4 (3)	69,4	9,05
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	101	35963	2	9,1	9,92
	contig26443:1:303:1 No Annotation	84	11655	1	12,9	4,72
D11	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	47	20178	2 (1)	9,7	4,84
	contig13035:1:170:1 No Annotation	42	6468	1	14,3	5,25
	contig24531:1:590:3 No Annotation	246	22259	4	23,5	4,65
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	220	33965	5	16,8	6,71
D12	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	219	55650	4	9,9	6,86
	contig00389:54:533:3 No Annotation	31	18426	1	6,9	4,95
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	74	33965	2	6,9	6,71
D13	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	61	55650	1	2,3	6,86
	contig08625:1:110:2 No Annotation	187	4172	4 (3)	69,4	9,05
	contig26443:1:303:1 No Annotation	136	11655	2	23,8	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	95	35963	2	9,1	9,92
D14	contig13035:1:170:1 No Annotation	41	6468	1	14,3	5,25
	contig08625:1:110:2 No Annotation	181	4172	4 (3)	69,4	9,05

## Supplementary

	contig26443:1:303:1 No Annotation	146	11655	2	23,8	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	101	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	46	20178	2 (1)	9,7	4,84
	contig13035:1:170:1 No Annotation	45	6468	1	14,3	5,25
	contig03062:1:281:2 No Annotation	38	11033	1	12,9	9,23
D15	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	190	29873	4	16	5,36
	contig26443:1:303:1 No Annotation	94	11655	1	12,9	4,72
	contig24360:1:563:3 No Annotation	73	21499	2 (1)	7	5
	contig13035:1:170:1 No Annotation	42	6468	1	14,3	5,25
	contig08625:1:110:2 No Annotation	36	4172	1	22,2	9,05
D16	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	263	29873	6	22,7	5,36
	contig24360:1:563:3 No Annotation	196	21499	6 (4)	40,6	5
	contig26443:1:303:1 No Annotation	75	11655	1	12,9	4,72
	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	45	25352	1	4,1	4,85
D17	contig24360:1:563:3 No Annotation	440	21499	10 (8)	54	5
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	379	29873	8 (7)	26,6	5,36
	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	152	25352	3	18,6	4,85
	contig02295:455:757:2 No Annotation	114	11684	2	36,6	6,82
	contig26443:1:303:1 No Annotation	80	11655	1	12,9	4,72
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	58	33965	2	6,9	6,71
D18	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	171	29873	4	15,2	5,36
	contig24360:1:563:3 No Annotation	99	21499	3 (2)	12,3	5
	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	43	25352	1	4,1	4,85
	contig13035:1:170:1 No Annotation	42	6468	1	14,3	5,25
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	39	33965	1	3	6,71
	contig24531:1:590:3 No Annotation	33	22259	1	6,1	4,65
D19	contig08625:1:110:2 No Annotation	163	4172	3	69,4	9,05
	contig26443:1:303:1 No Annotation	106	11655	2	23,8	4,72
	contig13035:1:170:1 No Annotation	51	6468	1	14,3	5,25
D20	contig13035:1:170:1 No Annotation	45	6468	1	14,3	5,25
	contig26443:1:303:1 No Annotation	37	11655	1	10,9	4,72
	contig08625:1:110:2 No Annotation	37	4172	1	22,2	9,05
D21	contig26443:1:303:1 No Annotation	61	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	34	6468	1	14,3	5,25
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	30	35963	1	3,2	9,92
D22	contig26443:1:303:1 No Annotation	73	11655	1	12,9	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	50	35963	1	3,2	9,92
	contig13035:1:170:1 No Annotation	33	6468	1	14,3	5,25
D23	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	64	56002	1	2,2	6,01
	contig26443:1:303:1 No Annotation	63	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	46	6468	1	14,3	5,25
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	45	35963	1	3,2	9,92

## Supplementary

D24	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	28	20178	1	9,7	4,84
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	185	56002	4	8,1	6,01
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	46	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	34	20178	1	9,7	4,84
D27	contig08625:1:110:2 No Annotation	163	4172	3	69,4	9,05
	contig26443:1:303:1 No Annotation	133	11655	3	43,6	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	90	35963	2	9,1	9,92
	contig13035:1:170:1 No Annotation	72	6468	2	57,1	5,25
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	49	56002	1	2,2	6,01
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	46	20178	2 (1)	9,7	4,84
	contig03062:1:281:2 No Annotation	33	11033	1	12,9	9,23
D28	contig26443:1:303:1 No Annotation	109	11655	2	23,8	4,72
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	80	56002	2	4,7	6,01
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	58	35963	1	3,2	9,92
	contig13035:1:170:1 No Annotation	43	6468	1	14,3	5,25
	contig24531:1:590:3 No Annotation	39	22259	1	7,1	4,65
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	37	20178	2 (1)	9,7	4,84
	contig08625:1:110:2 No Annotation	37	4172	1	22,2	9,05
D29	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	190	56002	4	10,5	6,01
	contig18438:8:349:2 No Annotation	77	12958	2	23	8,58
D30	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	309	56002	7 (6)	14,4	6,01
	contig18438:8:349:2 No Annotation	73	12958	2	23	8,58
D31	contig08625:1:110:2 No Annotation	147	4172	3	69,4	9,05
	contig26443:1:303:1 No Annotation	122	11655	2	23,8	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	95	35963	2	9,1	9,92
	contig13035:1:170:1 No Annotation	52	6468	1	14,3	5,25
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	37	20178	1	9,7	4,84
	contig18537:1:1312:2 No Annotation	28	49479	1	2,5	5,84
D32	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	315	55650	7 (2)	12,6	6,86
	contig18537:1:1312:2 No Annotation	284	49479	7	18,8	5,84
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	127	56002	2	4,7	6,01
	contig18438:8:349:2 No Annotation	107	12958	3	32,7	8,58
	contig26443:1:303:1 No Annotation	63	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	45	6468	1	14,3	5,25
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	39	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	36	20178	1	9,7	4,84
	contig08625:1:110:2 No Annotation	35	4172	1	22,2	9,05
	D33	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	452	56002	12 (10)	21,1
contig18438:8:349:2 No Annotation		83	12958	2	23	8,58
contig18537:1:1312:2 No Annotation		41	49479	1	2,7	5,84
D34	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	293	56002	8 (6)	16,8	6,01
	contig18438:8:349:2 No Annotation	128	12958	3	32,7	8,58

## Supplementary

	contig18291:688:825:1 similar to U4/U6 small nuclear ribonucleoprotein Prp4 (O43172) Evalue: 1e-04	28	5128	1	15,6	8,71
D35	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	297	56002	6	14	6,01
	contig18537:1:1312:2 No Annotation	105	49479	2	5,3	5,84
	contig18438:8:349:2 No Annotation	86	12958	2	23	8,58
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	47	55650	1	2,3	6,86
D36	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	357	56002	9 (7)	19,7	6,01
	contig18438:8:349:2 No Annotation	126	12958	3	32,7	8,58
D37	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	405	56002	8 (7)	15,2	6,01
	contig18438:8:349:2 No Annotation	130	12958	3	32,7	8,58
D38	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	184	56002	4	10,8	6,01
	contig18438:8:349:2 No Annotation	86	12958	2	23	8,58
	contig08625:1:110:2 No Annotation	71	4172	2	69,4	9,05
	contig26443:1:303:1 No Annotation	67	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	41	6468	1	14,3	5,25
D39	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	252	56002	7 (6)	15,6	6,01
	contig18438:8:349:2 No Annotation	81	12958	2	23	8,58
	contig26256:1:544:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-37	27	20475	1	6,6	5,13
D40	contig18537:1:1312:2 No Annotation	121	49479	3 (2)	5,7	5,84
	contig28231:1:506:3 No Annotation	78	19585	3 (2)	17,3	4,99
	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
D41	contig14499:1:464:3 similar to Alpha-2-macroglobulin-like protein 1 (A8K2U0) Evalue: 9e-05	222	17495	6 (5)	30,1	5,54
	contig14499:601:864:1 similar to CD109 antigen (Q6YHK3) Evalue: 1e-14	65	10000	1	12,5	5,37
	contig18537:1:1312:2 No Annotation	45	49479	1	2,1	5,84
D42	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	56	56002	1	2,4	6,01
	contig28231:1:506:3 No Annotation	45	19585	1	6	4,99
	contig18438:8:349:2 No Annotation	33	12958	1	8	8,58
	contig18537:1:1312:2 No Annotation	29	49479	1	2,1	5,84
D43	contig18537:1:1312:2 No Annotation	111	49479	3 (2)	5,7	5,84
	contig28231:1:506:3 No Annotation	84	19585	2	17,3	4,99
D44	contig18537:1:1312:2 No Annotation	93	49479	3 (2)	5,7	5,84
	contig28231:1:506:3 No Annotation	78	19585	2	17,9	4,99
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	40	35963	1	3,2	9,92
D45	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
D46	contig24910:1:199:2 No Annotation	32	7556	1	12,3	4,9
D47	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	49	20178	1	9,7	4,84
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	47	35963	1	3,2	9,92
	contig13035:1:170:1 No Annotation	41	6468	1	14,3	5,25
	contig26443:1:303:1 No Annotation	39	11655	1	10,9	4,72
	contig08625:1:110:2 No Annotation	37	4172	1	22,2	9,05
D48	contig18537:1:1312:2 No Annotation	98	49479	2	5,7	5,84
D49	contig18537:1:1312:2 No Annotation	121	49479	4 (2)	5,7	5,84
	contig28231:1:506:3 No Annotation	74	19585	3 (2)	17,3	4,99

## Supplementary

D50	contig18537:1:1312:2 No Annotation	43	49479	1	2,1	5,84
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	36	56002	1	5,1	6,01
D51	contig24531:1:590:3 No Annotation	91	22259	2	13,3	4,65
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	56	55650	1	2,3	6,86
D52	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	33	56002	1	2,4	6,01
D53	contig13124:1:1993:2 similar to Alpha-actinin-2 (Q3ZC55) Evalue: 0.0	494	77104	11 (10)	16,6	6,19
D55	contig24462:119:1723:2 similar to T-complex protein 1 subunit beta (P78371) Evalue: 0.0	211	58563	5	9,7	5,88
	contig24910:1:199:2 No Annotation	33	7556	1	12,3	4,9
D56	contig21701:200:1894:2 similar to Protein disulfide-isomerase A3 (P38657) Evalue: 1e-134	241	63808	6	12,1	6,63
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
D57	contig21701:200:1894:2 similar to Protein disulfide-isomerase A3 (P38657) Evalue: 1e-134	643	63808	14 (12)	23,2	6,63
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
D58	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	294	56002	5	11,4	6,01
	contig18438:8:349:2 No Annotation	106	12958	3	32,7	8,58
	contig26443:1:303:1 No Annotation	78	11655	1	12,9	4,72
D59	contig21701:200:1894:2 similar to Protein disulfide-isomerase A3 (P38657) Evalue: 1e-134	270	63808	8 (7)	14,2	6,63
D60	contig21701:200:1894:2 similar to Protein disulfide-isomerase A3 (P38657) Evalue: 1e-134	100	63808	3	5,7	6,63
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	82	56002	3 (2)	6,7	6,01
D61	contig25802:1:1389:1 similar to Enolase (Q27527) Evalue: 0.0	606	50818	10 (9)	26,2	6,25
D62	contig18438:8:349:2 No Annotation	199	12958	4	26,5	8,58
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	153	56002	5 (3)	6,7	6,01
D64	contig19251:1970:2808:2 similar to T-complex protein 1 subunit epsilon (P80316) Evalue: 5e-109	39	30483	1	3,9	6,02
	contig21701:200:1894:2 similar to Protein disulfide-isomerase A3 (P38657) Evalue: 1e-134	31	63808	1	1,6	6,63
D65	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	75	55650	1	2,9	6,86
D66	contig03109:97:1837:1 similar to Lamin Dm0 (P08928) Evalue: 4e-60	32	66260	1	1,7	5,33
D67	contig00798:1:1071:1 similar to Alpha-aminoadipic semialdehyde dehydrogenase (P49419) Evalue: 4e-131	102	39000	2	6,7	5,36
	contig18537:1:1312:2 No Annotation	30	49479	1	2,7	5,84
D68	contig00798:1:1071:1 similar to Alpha-aminoadipic semialdehyde dehydrogenase (P49419) Evalue: 4e-131	273	39000	6	20,4	5,36
	contig18205:1:513:1 similar to Alpha-aminoadipic semialdehyde dehydrogenase (Q9DBF1) Evalue: 1e-70	31	18619	1	6,5	8,59
D69	contig18438:8:349:2 No Annotation	36	12958	1	15	8,58
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	32	33965	1	3,3	6,71
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	30	56002	2 (1)	1,6	6,01
D72	contig24360:1:563:3 No Annotation	62	21499	2 (1)	7	5
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	60	29873	1	3,9	5,36
D74	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	135	29873	3	10,9	5,36
	contig24360:1:563:3 No Annotation	110	21499	2	13,9	5
	contig24910:1:199:2 No Annotation	41	7556	1	12,3	4,9
D76	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	108	29873	2	7,8	5,36
	contig24360:1:563:3 No Annotation	70	21499	2	12,3	5
D81	contig23341:69:1196:3 similar to Actin, alpha skeletal muscle (P68139) Evalue: 0.0	34	42370	1	2,9	5,75
D84	contig23953:1:1637:3 similar to Aldehyde dehydrogenase X, mitochondrial (P30837) Evalue: 0.0	459	59563	9	19,9	6,92
	contig24956:1:847:3 similar to Phosphoglycerate kinase (P51903) Evalue: 2e-108	64	30960	1	5	5,85

## Supplementary

D87	contig24774:1:1265:3 similar to Serpin B4 (P48594) Evalue: 2e-49	122	47575	3	6,7	6,2
	contig17746:1:1516:2 similar to 6-phosphogluconate dehydrogenase, decarboxylating (P41570) Evalue: 0.0	30	56657	1	1,8	8,32
D88	contig17807:1:460:2 similar to Glutamine synthetase (P51121) Evalue: 9e-67	238	16934	5 (4)	39,5	6,71
	contig01713:1:894:1 similar to Prostatic acid phosphatase (A6H730) Evalue: 2e-28	74	33942	1	4,4	5,98
	contig25577:1:379:3 similar to Glutamine synthetase (Q04831) Evalue: 4e-55	42	14379	1	6,4	5,65
	contig22889:238:502:1 similar to Glutamine synthetase (P15103) Evalue: 1e-27	33	9926	1	10,2	5,2
D89	contig00947:1:818:3 similar to Annexin A11 (P33477) Evalue: 6e-48	244	31607	6	27,7	5,48
	contig17838:1:476:2 similar to Annexin A6 (P79134) Evalue: 2e-08	138	18060	3	22,2	8,46
	contig20348:117:860:3 similar to Elongation factor 1-gamma (Q90YC0) Evalue: 6e-39	36	27237	1	4	8,39
	contig25021:205:1170:1 similar to D-3-phosphoglycerate dehydrogenase (O43175) Evalue: 6e-99	28	34855	1	3,1	5,84
D90	contig00947:1:818:3 similar to Annexin A11 (P33477) Evalue: 6e-48	368	31607	10 (9)	37,3	5,48
	contig17838:1:476:2 similar to Annexin A6 (P79134) Evalue: 2e-08	145	18060	3	22,2	8,46
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
D91	contig20348:117:860:3 similar to Elongation factor 1-gamma (Q90YC0) Evalue: 6e-39	131	27237	3	12,9	8,39
D92	contig01168:22:1775:1 similar to Trifunctional enzyme subunit alpha, mitochondrial (Q8BMS1) Evalue: 0.0	301	63836	7 (6)	9,9	9,17
	contig00142:113:1300:2 similar to Stomatatin-like protein 2 (Q99JB2) Evalue: 3e-115	113	43945	2	5,1	8,94
	contig01051:8:172:2 similar to Trifunctional enzyme subunit alpha, mitochondrial (Q64428) Evalue: 1e-12	83	6378	2	42,6	9,58
	contig18537:1:1312:2 No Annotation	42	49479	1	2,5	5,84
	contig22552:1:2159:2 similar to Peroxidase (A4IGL7) Evalue: 7e-54	41	84539	1	1,7	5,64
D95	contig25021:205:1170:1 similar to D-3-phosphoglycerate dehydrogenase (O43175) Evalue: 6e-99	116	34855	3 (2)	6,5	5,84
	contig17838:1:476:2 similar to Annexin A6 (P79134) Evalue: 2e-08	39	18060	1	7,6	8,46
	contig25734:1:558:1 similar to Eukaryotic translation initiation factor 3 subunit I (Q16K15) Evalue: 3e-51	30	20518	1	7,6	5,66
D96	contig23772:17:1069:2 similar to 26S proteasome non-ATPase regulatory subunit 13 (P84169) Evalue: 8e-65	133	40929	4	12,5	5,99
	contig17838:1:476:2 similar to Annexin A6 (P79134) Evalue: 2e-08	123	18060	3	22,2	8,46
	contig07413:1:1541:1 similar to 60 kDa heat shock protein, mitochondrial (P63038) Evalue: 0.0	80	55565	2	7	5,88
	contig18537:1:1312:2 No Annotation	33	49479	1	2,5	5,84
	contig26527:1:99:1 No Annotation	32	3883	1	31,2	4,59
	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	32	38936	1	2,9	8,26
D97	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	153	36658	4 (3)	10,7	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	134	26956	4 (3)	14,2	5,31
	contig10844:1:307:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 7e-25	68	10508	1	10,9	6,02
	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	68	38936	1	2,9	8,26
D98	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	164	38936	2	6,7	8,26
	contig25322:103:1317:1 similar to Malate/L-sulfolactate dehydrogenase (P16142) Evalue: 3e-60	122	43638	4 (3)	7,9	8,09
	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	70	22292	2	10	5,26
	contig25609:1:118:2 No Annotation	58	4434	1	31,6	4,87
	contig10844:1:307:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 7e-25	57	10508	1	10,9	6,02
	contig18449:1:1229:3 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 2e-87	49	44803	1	2,9	8,14
D99	contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	312	38936	5	17,2	8,26
	contig10844:1:307:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 7e-25	147	10508	2	23,8	6,02
	contig25609:1:118:2 No Annotation	119	4434	2	71,1	4,87
	contig25322:103:1317:1 similar to Malate/L-sulfolactate dehydrogenase (P16142) Evalue: 3e-60	91	43638	3 (2)	7,4	8,09

## Supplementary

	contig02901:61:934:1 No Annotation	60	33114	1	5,5	7,45
	contig24098:128:1165:2 similar to Fructose-1,6-bisphosphatase isozyme 2 (Q9Z1N1) Evalue: 1e-124	58	37883	1	3,2	6,31
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	43	35963	1	3,2	9,92
	contig25021:205:1170:1 similar to D-3-phosphoglycerate dehydrogenase (O43175) Evalue: 6e-99	29	34855	1	3,1	5,84
	contig18537:1:1312:2 No Annotation	28	49479	1	2,5	5,84
D100	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	179	22292	4	26,5	5,26
	contig18449:1:1229:3 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 2e-87	87	44803	1	2,9	8,14
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	64	29873	2 (1)	9	5,36
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	50	20178	1	9,7	4,84
	contig28231:1:506:3 No Annotation	43	19585	1	6	4,99
	contig25872:119:1225:2 similar to Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (O44451) Evalue: 1e-135	36	40625	1	3	6,12
	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	29	46596	1	3,6	7,71
D102	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	264	36658	6 (5)	22,6	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	251	26956	6 (5)	30	5,31
	contig18537:1:1312:2 No Annotation	220	49479	5	11,4	5,84
	contig07206:1:1594:2 No Annotation	47	58081	1	2,1	5,51
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	29	13132	1	9,3	5,46
D103	contig26710:1:1127:3 similar to Obg-like ATPase 1 (Q7ZWM6) Evalue: 1e-155	73	42366	2	7,2	6,08
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	53	36658	1	3,1	5,84
	contig23629:1:1130:3 similar to 28S ribosomal protein S31, mitochondrial (Q61733) Evalue: 3e-30	44	43306	1	2,9	7,21
	contig20348:117:860:3 similar to Elongation factor 1-gamma (Q90YC0) Evalue: 6e-39	42	27237	1	4	8,39
	contig17838:1:476:2 similar to Annexin A6 (P79134) Evalue: 2e-08	33	18060	1	7,6	8,46
	contig19059:1:2293:2 similar to Neprilysin-2 (O16796) Evalue: 2e-108	32	87708	1	1,2	6,31
D104	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	102	26956	3	14,2	5,31
	contig20348:117:860:3 similar to Elongation factor 1-gamma (Q90YC0) Evalue: 6e-39	58	27237	1	4	8,39
	contig18537:1:1312:2 No Annotation	42	49479	1	2,5	5,84
D105	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	599	64906	9	19,8	5,47
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	37	35963	1	3,2	9,92
D106	contig20348:117:860:3 similar to Elongation factor 1-gamma (Q90YC0) Evalue: 6e-39	90	27237	2	7,7	8,39
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	65	36658	2	5,3	5,84
	contig13035:1:170:1 No Annotation	51	6468	1	14,3	5,25
	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	39	64906	1	2,2	5,47
	contig08625:1:110:2 No Annotation	36	4172	1	22,2	9,05
	contig24244:268:651:1 No Annotation	35	14730	1	6,2	4,77
D107	contig04581:1:1472:3 similar to Tubulin alpha-4A chain (P68368) Evalue: 0.0	207	55209	5 (4)	11,2	5,25
	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	32	22292	1	5,5	5,26
D108	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	67	36658	2	6,6	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	30	13132	1	15,3	5,46
D109	contig24956:1:847:3 similar to Phosphoglycerate kinase (P51903) Evalue: 2e-108	181	30960	3	12,8	5,85
	contig25802:1:1389:1 similar to Enolase (Q27527) Evalue: 0.0	179	50818	2	5,8	6,25
D110	contig25921:1:1436:3 similar to Tubulin beta-1 chain (O17449) Evalue: 0.0	303	54166	7 (6)	16,8	4,92



## Supplementary

	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	164	22292	5 (3)	20	5,26
	contig18449:1:1229:3 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 2e-87	110	44803	2	5,9	8,14
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	97	29873	2 (1)	9	5,36
	contig04575:1:808:2 No Annotation	43	29692	1	5,6	5,71
D111	contig25872:119:1225:2 similar to Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (O44451) Evalue: 1e-135	437	40625	9 (8)	26,4	6,12
D118	contig18840:102:891:3 similar to Transforming growth factor-beta-induced protein ig-h3 (Q15582) Evalue: 4e-09	174	30016	4	11,4	5,53
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	106	35963	2	9,1	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	30	20178	1	9,7	4,84
D120	contig07785:1:816:1 No Annotation	58	30617	1	4,8	6,15
D121	contig26455:147:911:3 No Annotation	78	28119	1	7,1	5,92
D123	contig18941:155:934:2 No Annotation	65	27813	1	4,2	5,83
	contig24910:1:199:2 No Annotation	38	7556	1	12,3	4,9
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	32	35963	1	3,2	9,92
D124	contig18941:155:934:2 No Annotation	261	27813	6 (5)	30,1	5,83
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	57	35963	1	3,2	9,92
	contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	48	20178	1	9,7	4,84
D125	contig18941:155:934:2 No Annotation	79	27813	2	10,8	5,83
D128	contig18941:155:934:2 No Annotation	125	27813	3	14,7	5,83
	contig20272:1:245:1 similar to Neuronal acetylcholine receptor subunit beta-2 (P09484) Evalue: 3e-05	39	9227	1	13,6	5
D140	contig25886:1:136:2 No Annotation	172	5205	3	42,2	4,46
	contig20272:1:245:1 similar to Neuronal acetylcholine receptor subunit beta-2 (P09484) Evalue: 3e-05	148	9227	4 (3)	25,9	5
	contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	99	56051	2	4,8	5,38
	contig06986:298:1467:1 similar to Probable pyruvate dehydrogenase E1 component subunit alpha, mitochondrial (P52899) Evalue: 7e-140	77	43876	2	6,2	8,3
	contig25707:1:192:1 No Annotation	75	7595	1	18,8	4,82
	contig24441:1:655:2 similar to Hydroxyacylglutathione hydrolase, mitochondrial (Q6P963) Evalue: 1e-68	67	25041	1	5,5	6,06
	contig08799:1:1630:2 similar to Citrate synthase, mitochondrial (Q28DK1) Evalue: 0.0	28	60830	1	1,8	8,53
D153	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	118	56002	3	7,1	6,01
	contig18815:36:1291:3 similar to Tyrosine-protein phosphatase non-receptor type 4 (Q9WU22) Evalue: 4e-41	52	48010	1	3,3	9,13
D159	contig00858:452:928:2 similar to Peroxiredoxin-6 (Q5ZJF4) Evalue: 3e-45	446	17552	8	63,3	5,66
	contig21746:1:589:2 similar to Glutathione S-transferase 1 (P46436) Evalue: 6e-32	405	22033	9 (8)	34,9	5,33
	contig01555:212:841:2 similar to S-crystallin 4 (P27012) Evalue: 3e-25	127	24208	3	19,6	6,43
	contig20201:1:273:1 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 5e-24	120	9668	2	42,2	5,63
	contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	115	42823	2	8,4	5,27
	contig18321:1:789:3 similar to Chymotrypsin-like elastase family member 2A (P08419) Evalue: 3e-23	101	29034	2	8	5,56
	contig22415:1:289:3 similar to Trypsin gamma (Q9NRR2) Evalue: 2e-10	75	10316	1	15,8	6,75
	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	28	41570	1	5	6,6
D166	contig07785:1:816:1 No Annotation	75	30617	1	4,8	6,15
	contig01555:212:841:2 similar to S-crystallin 4 (P27012) Evalue: 3e-25	47	24208	1	3,8	6,43
	contig19529:448:736:1 similar to ATP synthase subunit d, mitochondrial (Q24251) Evalue: 6e-25	35	11005	1	10,4	8,89
	contig11153:105:404:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-22	30	11707	1	7	5,15
	contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	27	42088	1	1,9	6,79

## Supplementary

E3	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	34	25352	1	4,1	4,85
E4	contig26443:1:303:1 No Annotation	64	11655	1	12,9	4,72
	contig24360:1:563:3 No Annotation	60	21499	1	7	5
	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
E5	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	159	25352	3	25	4,85
	contig26443:1:303:1 No Annotation	149	11655	3	42,6	4,72
	contig24360:1:563:3 No Annotation	128	21499	2	13,9	5
	contig08625:1:110:2 No Annotation	121	4172	2	47,2	9,05
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	84	29873	2	7,8	5,36
	contig13035:1:170:1 No Annotation	43	6468	1	14,3	5,25
	contig02295:455:757:2 No Annotation	38	11684	1	21,8	6,82
	contig24910:1:199:2 No Annotation	36	7556	1	12,3	4,9
	contig09502:237:2105:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	29	68965	1	2,3	5,41
E6	contig08625:1:110:2 No Annotation	73	4172	2	69,4	9,05
	contig26443:1:303:1 No Annotation	61	11655	2	30,7	4,72
	contig13035:1:170:1 No Annotation	38	6468	1	14,3	5,25
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	34	29873	1	3,9	5,36
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	27	36658	1	3,4	5,84
E7	contig26443:1:303:1 No Annotation	186	11655	5 (4)	43,6	4,72
	contig08625:1:110:2 No Annotation	179	4172	3	69,4	9,05
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	140	33965	3	9,2	6,71
	contig24531:1:590:3 No Annotation	123	22259	3	23	4,65
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	111	29873	2	8,6	5,36
	contig13035:1:170:1 No Annotation	45	6468	1	14,3	5,25
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	42	24857	1	10,6	4,86
	contig24360:1:563:3 No Annotation	29	21499	1	7	5
E8	contig26443:1:303:1 No Annotation	132	11655	3	43,6	4,72
	contig13035:1:170:1 No Annotation	42	6468	1	14,3	5,25
	contig08625:1:110:2 No Annotation	41	4172	1	22,2	9,05
	contig24531:1:590:3 No Annotation	32	22259	1	9,7	4,65
E9	contig26443:1:303:1 No Annotation	238	11655	6 (5)	44,6	4,72
	contig08625:1:110:2 No Annotation	175	4172	4 (3)	69,4	9,05
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	155	56002	3	9,7	6,01
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	127	24857	5 (3)	22,7	4,86
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	122	29873	4 (3)	14,1	5,36
	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	110	25352	3	15,5	4,85
	contig13035:1:170:1 No Annotation	84	6468	2	57,1	5,25
	contig03062:1:281:2 No Annotation	69	11033	2 (1)	12,9	9,23
	contig02295:455:757:2 No Annotation	57	11684	2	23,8	6,82
contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	44	64906	1	1,6	5,47	
E10	contig24360:1:563:3 No Annotation	275	21499	9 (6)	40,6	5

## Supplementary

	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	231	29873	7 (5)	18,4	5,36
	contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	129	25352	3	21,8	4,85
	contig02295:455:757:2 No Annotation	126	11684	3 (2)	36,6	6,82
	contig08625:1:110:2 No Annotation	109	4172	2	47,2	9,05
	contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	83	33965	2	5,9	6,71
	contig26443:1:303:1 No Annotation	81	11655	1	12,9	4,72
	contig24531:1:590:3 No Annotation	44	22259	1	6,1	4,65
	contig13035:1:170:1 No Annotation	43	6468	1	14,3	5,25
E11	contig26443:1:303:1 No Annotation	106	11655	2	23,8	4,72
	contig08625:1:110:2 No Annotation	66	4172	2	72,2	9,05
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	55	56002	1	2,4	6,01
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	49	29873	1	3,9	5,36
	contig13035:1:170:1 No Annotation	45	6468	1	14,3	5,25
	contig24531:1:590:3 No Annotation	34	22259	1	7,1	4,65
E12	contig26443:1:303:1 No Annotation	101	11655	3	30,7	4,72
	contig08625:1:110:2 No Annotation	77	4172	2	25	9,05
	contig13035:1:170:1 No Annotation	45	6468	1	14,3	5,25
	contig24910:1:199:2 No Annotation	35	7556	1	12,3	4,9
	contig02107:1:417:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-35	34	16121	1	7,9	8,76
E14	contig13035:1:170:1 No Annotation	51	6468	1	14,3	5,25
E16	contig26443:1:303:1 No Annotation	120	11655	2	23,8	4,72
	contig14962:1:769:2 similar to Myosin heavy chain, muscle (P05661) Evalue: 6e-58	66	29584	2	8,6	5,1
	contig15494:1:257:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 8e-18	61	9528	1	14,1	4,83
	contig13035:1:170:1 No Annotation	43	6468	1	14,3	5,25
	contig08625:1:110:2 No Annotation	38	4172	1	22,2	9,05
E18	contig08625:1:110:2 No Annotation	158	4172	3	69,4	9,05
	contig14962:1:769:2 similar to Myosin heavy chain, muscle (P05661) Evalue: 6e-58	52	29584	1	4,3	5,1
	contig01519:1:1386:1 similar to CD109 antigen (Q8R422) Evalue: 2e-80	52	51074	1	2,6	7,78
	contig13035:1:170:1 No Annotation	51	6468	1	14,3	5,25
	contig24360:1:563:3 No Annotation	47	21499	1	7	5
	contig26443:1:303:1 No Annotation	43	11655	1	10,9	4,72
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	31	24857	1	4,2	4,86
E19	contig26443:1:303:1 No Annotation	117	11655	2	23,8	4,72
	contig15494:1:257:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 8e-18	46	9528	1	14,1	4,83
	contig14962:1:769:2 similar to Myosin heavy chain, muscle (P05661) Evalue: 6e-58	45	29584	1	4,3	5,1
	contig08625:1:110:2 No Annotation	41	4172	1	22,2	9,05
	contig13035:1:170:1 No Annotation	41	6468	1	14,3	5,25
E42	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	340	36658	8 (6)	24,8	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	336	26956	9 (6)	32,9	5,31
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	197	13132	3	33,9	5,46
	contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	128	56051	2	4,6	5,38
E43	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	493	36658	11 (8)	39,2	5,84

## Supplementary

	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	475	26956	12 (8)	52,1	5,31
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	236	13132	5 (3)	33,9	5,46
	contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	115	56051	2	4,4	5,38
	contig11420:186:337:3 similar to Actin (Q9UVX4) Evalue: 1e-25	56	5953	2 (1)	32	5,44
	contig24910:1:199:2 No Annotation	34	7556	1	12,3	4,9
E44	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	385	26956	9 (7)	39,6	5,31
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	385	36658	8 (7)	29,8	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	216	13132	5 (3)	33,9	5,46
E45	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	74	56002	2	6,7	6,01
	contig18438:8:349:2 No Annotation	30	12958	1	15	8,58
	contig00124:1:1376:3 similar to Eukaryotic initiation factor 4A-II (Q8JFP1) Evalue: 2e-166	29	52397	1	2,2	5,75
E46	contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	198	56051	5	11	5,38
E47	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	255	13132	5 (4)	42,4	5,46
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	233	36658	5 (4)	15,7	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	218	26956	5 (4)	20,8	5,31
	contig26159:38:948:2 similar to Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial (Q9Z2I8) Evalue: 1e-80	32	34297	1	4	6,04
E48	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	381	26956	11 (7)	39,6	5,31
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	379	36658	10 (7)	29,8	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	325	13132	6 (4)	42,4	5,46
	contig11420:186:337:3 similar to Actin (Q9UVX4) Evalue: 1e-25	41	5953	1	32	5,44
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
E49	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	334	26956	10 (6)	39,6	5,31
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	276	36658	9 (6)	29,8	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	167	13132	4 (3)	33,9	5,46
	contig04768:1:376:2 No Annotation	86	14183	2	28,2	6
	contig24910:1:199:2 No Annotation	34	7556	1	12,3	4,9
	contig24111:1:454:2 No Annotation	28	17747	1	5,3	4,69
E50	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	247	36658	5 (4)	18,5	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	221	13132	5 (4)	42,4	5,46
	contig11420:186:337:3 similar to Actin (Q9UVX4) Evalue: 1e-25	34	5953	1	32	5,44
E51	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	307	36658	6 (5)	22,3	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	296	26956	6 (5)	29,6	5,31
	contig00854:1:1427:3 similar to Endoplasmin (Q95M18) Evalue: 1e-143	190	54510	3	7,6	5,43
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	54	13132	2 (1)	15,3	5,46
E52	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	84	36658	2	8,2	5,84
	contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	51	14492	1	7,8	4,82
	contig19345:270:876:3 No Annotation	38	23208	1	4,5	5,13
E53	contig05979:197:1027:2 similar to 4-hydroxyphenylpyruvate dioxygenase (Q5BKL0) Evalue: 2e-108	335	31475	7	27,4	5,36
	contig05012:1:318:1 similar to 4-hydroxyphenylpyruvate dioxygenase (P32754) Evalue: 2e-40	66	12251	1	12,4	5,16
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	41	36658	1	3,1	5,84
E54	contig18438:8:349:2 No Annotation	31	12958	1	15	8,58

## Supplementary

	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	29	56002	1	1,6	6,01
E55	contig03976:126:2186:3 similar to Chaperone protein dnaK (A6T4F4) Evalue: 0.0	364	75009	10 (9)	13,8	6,02
	contig26159:38:948:2 similar to Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial (Q9Z2I8) Evalue: 1e-80	85	34297	2	7,3	6,04
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	56	36658	2	6,6	5,84
E56	contig25802:1:1389:1 similar to Enolase (Q27527) Evalue: 0.0	450	50818	9 (8)	24,7	6,25
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	215	36658	5 (4)	17,9	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	191	26956	5 (4)	23,8	5,31
	contig08625:1:110:2 No Annotation	160	4172	3	69,4	9,05
	contig26443:1:303:1 No Annotation	123	11655	2	23,8	4,72
	contig08235:860:1596:2 No Annotation	90	27856	2	13,1	5,65
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	76	13132	2	24,6	5,46
	contig13035:1:170:1 No Annotation	52	6468	1	14,3	5,25
E57	contig25802:1:1389:1 similar to Enolase (Q27527) Evalue: 0.0	508	50818	8	22,1	6,25
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	228	36658	7 (5)	24,5	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	216	26956	6 (5)	32,5	5,31
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	78	13132	2	24,6	5,46
	contig03109:97:1837:1 similar to Lamin Dm0 (P08928) Evalue: 4e-60	63	66260	1	1,7	5,33
	contig26443:1:303:1 No Annotation	58	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	52	6468	1	14,3	5,25
	contig20608:1:663:2 similar to Spectrin alpha chain (P13395) Evalue: 2e-53	46	26036	1	4,5	6,38
	contig04581:1:1472:3 similar to Tubulin alpha-4A chain (P68368) Evalue: 0.0	35	55209	1	2,9	5,25
	contig07385:1:906:1 similar to 40S ribosomal protein SA (B5FXT6) Evalue: 5e-100	29	33819	1	2,7	5,93
E58	contig08625:1:110:2 No Annotation	172	4172	3	69,4	9,05
	contig26443:1:303:1 No Annotation	108	11655	2	23,8	4,72
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	47	36658	2 (1)	3,4	5,84
	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	33	13132	1	9,3	5,46
E59	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	143	36658	3	10,7	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	123	26956	3	14,2	5,31
	contig08625:1:110:2 No Annotation	82	4172	1	44,4	9,05
	contig26443:1:303:1 No Annotation	78	11655	1	12,9	4,72
	contig25802:1:1389:1 similar to Enolase (Q27527) Evalue: 0.0	49	50818	1	2,6	6,25
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	45	13132	1	9,3	5,46
	contig13035:1:170:1 No Annotation	43	6468	1	14,3	5,25
E60	contig26443:1:303:1 No Annotation	103	11655	2	23,8	4,72
	contig08625:1:110:2 No Annotation	73	4172	2	69,4	9,05
	contig08235:860:1596:2 No Annotation	59	27856	1	4,1	5,65
	contig13035:1:170:1 No Annotation	44	6468	1	14,3	5,25
E61	contig26443:1:303:1 No Annotation	41	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
	contig08625:1:110:2 No Annotation	38	4172	1	22,2	9,05

## Supplementary

E63	contig26443:1:303:1 No Annotation	69	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	39	6468	1	14,3	5,25
	contig08625:1:110:2 No Annotation	37	4172	1	22,2	9,05
	contig06721:1:451:2 No Annotation	33	16943	1	10,7	4,73
E64	contig25094:2604:4781:3 similar to Heat shock protein HSP 90-alpha (Q76LV2) Evalue: 0.0	176	83855	5 (4)	8,4	5,01
	contig08625:1:110:2 No Annotation	140	4172	2	66,7	9,05
	contig26443:1:303:1 No Annotation	119	11655	2	23,8	4,72
	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	50	59942	1	1,9	4,95
	contig13035:1:170:1 No Annotation	44	6468	1	14,3	5,25
	contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	43	56051	1	2,5	5,38
	contig04581:1:1472:3 similar to Tubulin alpha-4A chain (P68368) Evalue: 0.0	39	55209	1	2,9	5,25
E65	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	94	56002	2	5,1	6,01
	contig05871:1:393:1 No Annotation	78	14042	1	8,5	4,93
	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	72	59942	1	1,9	4,95
	contig26443:1:303:1 No Annotation	53	11655	1	12,9	4,72
	contig08625:1:110:2 No Annotation	52	4172	1	44,4	9,05
	contig14962:1:769:2 similar to Myosin heavy chain, muscle (P05661) Evalue: 6e-58	45	29584	1	4,3	5,1
	contig18438:8:349:2 No Annotation	37	12958	1	15	8,58
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	36	36658	1	3,1	5,84
E66	contig18438:8:349:2 No Annotation	136	12958	3	32,7	8,58
	contig26443:1:303:1 No Annotation	70	11655	1	12,9	4,72
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	47	56002	2 (1)	5,1	6,01
	contig06721:1:451:2 No Annotation	33	16943	1	10,7	4,73
E67	contig25094:2604:4781:3 similar to Heat shock protein HSP 90-alpha (Q76LV2) Evalue: 0.0	110	83855	2	3,4	5,01
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	77	36658	2	6,6	5,84
	contig25994:1:1190:1 No Annotation	56	45325	1	2,3	5,95
E68	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	159	36658	4 (3)	10,7	5,84
	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	140	26956	4 (3)	14,2	5,31
	contig24968:1:1457:3 similar to Tubulin beta-1 chain (O17449) Evalue: 0.0	124	55045	2	4,3	4,89
	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	41	22292	1	5,5	5,26
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	29	13132	1	9,3	5,46
	contig18449:1:1229:3 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 2e-87	28	44803	1	2,9	8,14
E69	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	71	36658	2	6,6	5,84
	contig15928:1:732:1 similar to Tubulin beta chain (P11833) Evalue: 9e-93	52	27511	1	4,1	6,24
	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	31	29873	1	9	5,36
E70	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	270	26956	7 (5)	30,4	5,31
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	257	36658	6 (5)	22,9	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	99	13132	3 (2)	24,6	5,46
	contig26271:55:1587:1 similar to ATP-binding cassette sub-family E member 1 (P61222) Evalue: 0.0	75	58342	1	2,3	7,01
	contig26443:1:303:1 No Annotation	61	11655	1	12,9	4,72
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	41	35963	1	3,2	9,92
	contig25994:1:1190:1 No Annotation	38	45325	1	2,3	5,95

## Supplementary

E71	contig05711:251:901:2 similar to Pyruvate kinase isozyme M1 (P11979) Evalue: 7e-73	186	23916	3	16,6	5,05
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	65	56002	2	6,7	6,01
	contig25994:1:1190:1 No Annotation	55	45325	1	2,3	5,95
E72	contig24199:214:1227:1 similar to Uncharacterized protein ZK1073.1 (O02485) Evalue: 2e-70	32	38027	1	3,6	6,33
	contig26819:1:509:2 similar to Talin-2 (Q9Y4G6) Evalue: 2e-50	30	19612	1	5,3	9,21
E73	contig02624:1:558:1 similar to Inorganic pyrophosphatase (Q9D819) Evalue: 1e-52	166	21323	2	14,6	4,81
	contig18740:1:458:1 similar to Inorganic pyrophosphatase (O77460) Evalue: 1e-31	72	17692	1	7,2	9,5
	contig24199:214:1227:1 similar to Uncharacterized protein ZK1073.1 (O02485) Evalue: 2e-70	41	38027	1	3,6	6,33
	contig24910:1:199:2 No Annotation	32	7556	1	12,3	4,9
E74	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	280	59942	5	12,3	4,95
	contig27897:1:262:2 similar to Coatomer subunit epsilon (Q28104) Evalue: 8e-16	40	10041	1	16,3	4,95
E76	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	312	59942	5	12,3	4,95
	contig25094:2604:4781:3 similar to Heat shock protein HSP 90-alpha (Q76LV2) Evalue: 0.0	28	83855	1	1,5	5,01
E77	contig07385:1:906:1 similar to 40S ribosomal protein SA (B5FXT6) Evalue: 5e-100	117	33819	2	7	5,93
E79	contig02295:455:757:2 No Annotation	48	11684	1	21,8	6,82
E80	contig18129:136:1026:1 similar to Cathepsin Z (P05689) Evalue: 2e-100	102	33940	2	7,8	6,58
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	28	24857	1	10,2	4,86
E81	contig00650:1:1097:3 similar to S-adenosylmethionine synthetase isoform type-2 (Q4R924) Evalue: 2e-149	38	40698	1	5,8	6,19
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
E83	contig22702:1:727:3 similar to 26S proteasome non-ATPase regulatory subunit 8 (Q5RE15) Evalue: 2e-48	129	27735	3	14,1	5,41
E85	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	32	36658	1	3,1	5,84
E87	contig23622:1:2311:2 similar to Glycogen phosphorylase (Q9PKE6) Evalue: 0.0	32	88376	1	1	6,18
E88	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	116	59942	2	4,2	4,95
E89	contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	372	56051	8 (6)	16,8	5,38
	contig21183:1:1353:1 similar to Rab GDP dissociation inhibitor beta (P50395) Evalue: 4e-165	178	50742	4	9,8	6,26
E90	contig02692:53:1738:2 similar to Basement membrane proteoglycan (Q06561) Evalue: 6e-05	189	64773	4	8,7	5,14
E91	contig17623:1:1091:1 similar to Pyruvate carboxylase 1 (O17732) Evalue: 1e-91	39	40299	1	2,8	5,16
	contig05759:1:242:1 No Annotation	36	8815	1	10	9,26
	contig11404:1768:2695:2 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit 27 (Q6GNR9) Evalue: 1e-87	27	34474	1	5,8	6,79
E92	contig28056:1:780:1 similar to Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (Q07536) Evalue: 2e-116	271	27837	6 (5)	20,5	5,61
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	30	36658	1	3,1	5,84
E93	contig28056:1:780:1 similar to Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (Q07536) Evalue: 2e-116	192	27837	3	15,1	5,61
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	43	36658	1	3,1	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	32	13132	1	9,3	5,46
E94	contig23815:73:357:1 No Annotation	99	10301	3	33,7	3,91
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	36	36658	1	3,1	5,84
E96	contig04069:2305:3063:1 No Annotation	27	27925	1	3,6	6,6
E97	contig25359:2854:3612:2 similar to Uncharacterized methyltransferase GK2543 (Q5KWV8) Evalue: 5e-05	201	28870	4	15,1	5,44
E98	contig28039:246:1925:3 similar to Thioredoxin reductase 1, cytoplasmic (Q16881) Evalue: 1e-165	50	61478	1	2	5,81
	contig23591:1:328:2 similar to Plasminogen (P06868) Evalue: 4e-11	42	11694	1	11,1	8,35

## Supplementary

	contig18537:1:1312:2 No Annotation	29	49479	1	2,1	5,84
E99	contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	120	26956	4 (3)	14,2	5,31
E100	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	80	36658	2	6,6	5,84
	contig18219:1:768:1 similar to Proteasome subunit alpha type-3 (P25788) Evalue: 1e-89	35	28291	1	3,9	5,6
F1	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	779	59942	17 (14)	30	4,95
	contig13035:1:170:1 No Annotation	36	6468	1	14,3	5,25
F2	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	847	59942	23 (15)	33,8	4,95
F3	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	1057	59942	27 (18)	39,6	4,95
F6	contig26443:1:303:1 No Annotation	57	11655	1	12,9	4,72
	contig15928:1:732:1 similar to Tubulin beta chain (P11833) Evalue: 9e-93	39	27511	1	4,1	6,24
F7	contig25921:1:1436:3 similar to Tubulin beta-1 chain (O17449) Evalue: 0.0	53	54166	1	2,3	4,92
	contig26443:1:303:1 No Annotation	47	11655	1	12,9	4,72
F8	contig18537:1:1312:2 No Annotation	45	49479	1	2,1	5,84
	contig28231:1:506:3 No Annotation	27	19585	1	6	4,99
F9	contig17475:1:1628:3 similar to Mitochondrial-processing peptidase subunit beta (Q3SZ71) Evalue: 7e-169	127	61434	3	5,9	6,16
	contig26443:1:303:1 No Annotation	67	11655	1	12,9	4,72
	contig18537:1:1312:2 No Annotation	46	49479	1	2,1	5,84
	contig28231:1:506:3 No Annotation	35	19585	1	6	4,99
F11	contig28231:1:506:3 No Annotation	69	19585	2	17,3	4,99
	contig18537:1:1312:2 No Annotation	35	49479	1	2,1	5,84
F12	contig02760:1:1133:2 similar to Endoplasmin (P08113) Evalue: 2e-101	50	42740	1	3,2	4,89
	contig00854:1:1427:3 similar to Endoplasmin (Q95M18) Evalue: 1e-143	31	54510	1	3	5,43
F13	contig02760:1:1133:2 similar to Endoplasmin (P08113) Evalue: 2e-101	77	42740	1	3,2	4,89
F14	contig18504:154:1359:1 similar to Calreticulin (Q4R6K8) Evalue: 4e-127	572	46442	10	30,9	4,66
	contig24910:1:199:2 No Annotation	32	7556	1	12,3	4,9
F15	contig18022:1:620:3 similar to Nucleosome assembly protein 1-like 1 (Q28EB4) Evalue: 5e-20	60	23465	2	15,5	5,1
F17	contig06212:1:430:3 No Annotation	63	15630	1	8,5	6,45
	contig06721:1:451:2 No Annotation	48	16943	1	10,7	4,73
F19	contig06212:1:430:3 No Annotation	83	15630	2	24,6	6,45
	contig06721:1:451:2 No Annotation	53	16943	1	10,7	4,73
F20	contig02386:1:407:3 similar to 78 kDa glucose-regulated protein (Q90593) Evalue: 9e-42	36	15260	1	6	4,45
	contig15471:1:392:1 similar to Heat shock 70 kDa protein A (P09446) Evalue: 2e-54	33	14248	1	8,5	4,99
F21	contig26153:1:1449:2 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	123	53380	3	7,5	6,01
	contig02386:1:407:3 similar to 78 kDa glucose-regulated protein (Q90593) Evalue: 9e-42	82	15260	2	14,9	4,45
F22	contig26153:1:1449:2 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	55	53380	1	2,1	6,01
	contig02386:1:407:3 similar to 78 kDa glucose-regulated protein (Q90593) Evalue: 9e-42	36	15260	1	6	4,45
F23	contig15471:1:392:1 similar to Heat shock 70 kDa protein A (P09446) Evalue: 2e-54	43	14248	1	8,5	4,99
	contig02386:1:407:3 similar to 78 kDa glucose-regulated protein (Q90593) Evalue: 9e-42	36	15260	1	6	4,45
F24	contig25094:2604:4781:3 similar to Heat shock protein HSP 90-alpha (Q76LV2) Evalue: 0.0	305	83855	7 (6)	10,2	5,01
F25	contig25094:2604:4781:3 similar to Heat shock protein HSP 90-alpha (Q76LV2) Evalue: 0.0	401	83855	10 (8)	11,4	5,01
F26	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	76	56002	2	6,7	6,01
	contig18438:8:349:2 No Annotation	31	12958	1	15	8,58



## Supplementary

F27	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	281	59942	6	13,8	4,95
	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	268	46522	6 (5)	12,3	6,07
	contig07914:1:483:1 similar to Heat shock 70 kDa protein cognate 4 (Q9U639) Evalue: 9e-73	90	18328	2	11,9	4,86
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	63	36658	1	5	5,84
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	41	13132	1	9,3	5,46
F28	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	212	13132	6 (5)	51,7	5,46
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	141	36658	5 (4)	18,2	5,84
F29	contig00854:1:1427:3 similar to Endoplasmic (Q95M18) Evalue: 1e-143	133	54510	2	4,9	5,43
F30	contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	27	46522	1	3,3	6,07
F31	contig02113:1:319:2 No Annotation	126	10652	2	21,9	5,84
	contig00574:89:529:2 No Annotation	126	15521	2	17	5,5
	contig09723:1:288:1 No Annotation	41	10234	1	22,9	4,79
	contig13035:1:170:1 No Annotation	35	6468	1	14,3	5,25
	contig18068:1:139:2 No Annotation	31	5065	1	22,2	4,7
F32	contig00574:89:529:2 No Annotation	141	15521	2	17	5,5
	contig02113:1:319:2 No Annotation	124	10652	2	22,9	5,84
	contig09723:1:288:1 No Annotation	52	10234	1	22,9	4,79
	contig18068:1:139:2 No Annotation	33	5065	1	22,2	4,7
	contig26159:38:948:2 similar to Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial (Q9Z2I8) Evalue: 1e-80	33	34297	1	3,3	6,04
	contig06721:1:451:2 No Annotation	28	16943	1	10,7	4,73
F33	contig09723:1:288:1 No Annotation	109	10234	3 (2)	46,9	4,79
	contig26443:1:303:1 No Annotation	61	11655	1	12,9	4,72
	contig06721:1:451:2 No Annotation	48	16943	2 (1)	10,7	4,73
F34	contig09723:1:288:1 No Annotation	98	10234	2	46,9	4,79
	contig06721:1:451:2 No Annotation	51	16943	1	10,7	4,73
	contig26443:1:303:1 No Annotation	41	11655	1	12,9	4,72
F36	contig22479:1:399:3 similar to Tropomyosin (Q95VA8) Evalue: 1e-38	259	15276	5 (4)	30,3	4,71
	contig06212:1:430:3 No Annotation	159	15630	2	24,6	6,45
F37	contig06212:1:430:3 No Annotation	56	15630	2 (1)	15,5	6,45
F38	contig18504:154:1359:1 similar to Calreticulin (Q4R6K8) Evalue: 4e-127	233	46442	4	9,5	4,66
F40	contig01202:555:721:3 No Annotation	48	6398	1	29,1	4,94
	contig24910:1:199:2 No Annotation	37	7556	1	12,3	4,9
F41	contig01202:555:721:3 No Annotation	33	6398	1	29,1	4,94
F42	contig25843:96:1024:3 similar to Eukaryotic translation initiation factor 2 subunit 1 (Q5R493) Evalue: 4e-88	265	35454	5	16,8	5,26
	contig26159:38:948:2 similar to Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial (Q9Z2I8) Evalue: 1e-80	130	34297	3	11,9	6,04
	contig26443:1:303:1 No Annotation	46	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
	contig22681:1:150:2 similar to Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial (Q9Z2I8) Evalue: 6e-14	35	5196	1	18,4	5
F43	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	452	59942	9 (7)	14,5	4,95
F45	contig07385:1:906:1 similar to 40S ribosomal protein SA (B5FXT6) Evalue: 5e-100	96	33819	2	6,6	5,93

## Supplementary

F46	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	92	59942	3	6,6	4,95
	contig07385:1:906:1 similar to 40S ribosomal protein SA (B5FXT6) Evalue: 5e-100	45	33819	1	4	5,93
	contig26477:1:1199:3 similar to Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 (O45040) Evalue: 0.0	45	45019	1	3	6,47
	contig06721:1:451:2 No Annotation	38	16943	1	10,7	4,73
F49	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	363	59942	11 (9)	15,3	4,95
	contig21354:1:133:1 similar to Tropomyosin-2 (Q1HPQ0) Evalue: 3e-05	27	4885	1	22,7	4,45
F51	contig06721:1:451:2 No Annotation	58	16943	1	10,7	4,73
	contig06212:1:430:3 No Annotation	50	15630	1	8,5	6,45
F52	contig06721:1:451:2 No Annotation	48	16943	1	10,7	4,73
	contig06212:1:430:3 No Annotation	45	15630	1	8,5	6,45
F53	contig22479:1:399:3 similar to Tropomyosin (Q95VA8) Evalue: 1e-38	33	15276	1	9,1	4,71
F54	contig01202:555:721:3 No Annotation	62	6398	1	29,1	4,94
	contig06721:1:451:2 No Annotation	60	16943	1	10,7	4,73
	contig06212:1:430:3 No Annotation	59	15630	1	8,5	6,45
	contig18379:1:612:3 No Annotation	36	23288	1	4,9	4,95
F55	contig22765:1:560:3 similar to CD109 antigen (Q6YHK3) Evalue: 3e-16	56	21059	2	13	4,47
F56	contig03691:1:1595:3 similar to Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial (Q9Z2I9) Evalue: 3e-155	59	59462	1	1,9	5,43
	contig18379:1:612:3 No Annotation	36	23288	1	4,9	4,95
F57	contig07413:1:1541:1 similar to 60 kDa heat shock protein, mitochondrial (P63038) Evalue: 0.0	63	55565	1	2,3	5,88
F58	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	31	36658	1	3,1	5,84
F59	contig07385:1:906:1 similar to 40S ribosomal protein SA (B5FXT6) Evalue: 5e-100	30	33819	1	4	5,93
F61	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	32	36658	1	3,1	5,84
F62	contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evalue: 6e-144	201	59942	3	7,4	4,95
F63	contig22343:1:591:1 similar to Tumor protein D52 (P55327) Evalue: 1e-08	49	21345	1	7,1	5,5
F64	contig06212:1:430:3 No Annotation	130	15630	2	24,6	6,45
	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	43	13132	1	9,3	5,46
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	41	36658	1	3,1	5,84
F65	contig24625:236:982:2 similar to 14-3-3 protein zeta (Q2F637) Evalue: 2e-123	255	28345	5	21,8	4,84
	contig06212:1:430:3 No Annotation	37	15630	1	8,5	6,45
	contig24910:1:199:2 No Annotation	31	7556	1	12,3	4,9
F66	contig01202:555:721:3 No Annotation	52	6398	1	29,1	4,94
	contig06212:1:430:3 No Annotation	50	15630	1	8,5	6,45
	contig06721:1:451:2 No Annotation	48	16943	1	10,7	4,73
	contig25843:96:1024:3 similar to Eukaryotic translation initiation factor 2 subunit 1 (Q5R493) Evalue: 4e-88	45	35454	1	3,2	5,26
F67	contig02032:933:1673:2 similar to Eukaryotic translation initiation factor 6 (P56537) Evalue: 2e-115	119	26874	2	13	4,67
	contig01202:555:721:3 No Annotation	52	6398	1	29,1	4,94
	contig06212:1:430:3 No Annotation	51	15630	1	8,5	6,45
	contig06721:1:451:2 No Annotation	43	16943	1	10,7	4,73
F68	contig01202:555:721:3 No Annotation	53	6398	1	29,1	4,94
	contig06721:1:451:2 No Annotation	51	16943	1	10,7	4,73
F69	contig06212:1:430:3 No Annotation	70	15630	1	8,5	6,45

## Supplementary

	contig06721:1:451:2 No Annotation	65	16943	1	10,7	4,73
	contig01202:555:721:3 No Annotation	62	6398	1	29,1	4,94
	contig18321:1:789:3 similar to Chymotrypsin-like elastase family member 2A (P08419) Evalue: 3e-23	42	29034	1	3,4	5,56
F70	contig06721:1:451:2 No Annotation	76	16943	1	10,7	4,73
	contig06212:1:430:3 No Annotation	54	15630	1	8,5	6,45
F71	contig06212:1:430:3 No Annotation	65	15630	1	8,5	6,45
	contig01202:555:721:3 No Annotation	59	6398	1	29,1	4,94
F72	contig06212:1:430:3 No Annotation	65	15630	1	8,5	6,45
	contig01202:555:721:3 No Annotation	43	6398	1	29,1	4,94
F73	contig02240:94:701:1 similar to Glutathione-requiring prostaglandin D synthase (O73888) Evalue: 2e-13	154	23181	3	19,3	4,6
	contig06721:1:451:2 No Annotation	37	16943	1	10,7	4,73
F74	contig20875:1:776:3 similar to FK506-binding protein 7 (Q5RET2) Evalue: 1e-39	35	29026	1	5,1	5,25
F75	contig21335:1:161:1 similar to Translationally-controlled tumor protein homolog (Q5MIP6) Evalue: 3e-15	86	5844	1	22,6	4,13
	contig00873:1:333:1 similar to Translationally-controlled tumor protein homolog (Q60FS1) Evalue: 1e-32	59	12655	1	9,1	5,66
F80	contig08542:152:670:2 similar to Probable myosin regulatory light chain (Q09510) Evalue: 4e-50	373	18848	8 (6)	39,5	4,96
	contig24910:1:199:2 No Annotation	39	7556	1	12,3	4,9
	contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	33	42823	1	3,4	5,27
F81	contig08542:152:670:2 similar to Probable myosin regulatory light chain (Q09510) Evalue: 4e-50	424	18848	8 (7)	45,3	4,96
	contig17572:1:760:3 similar to Cathepsin L1 (Q9GKL8) Evalue: 1e-59	98	28921	1	5,6	6,64
	contig23523:1:350:3 similar to Cathepsin L (Q95029) Evalue: 2e-46	55	12510	1	12,2	4,61
	contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	50	42823	1	2,9	5,27
F83	contig08542:152:670:2 similar to Probable myosin regulatory light chain (Q09510) Evalue: 4e-50	183	18848	4 (3)	17,4	4,96
	contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	149	42823	3	11	5,27
F88	contig16627:471:1223:3 similar to Myosin, essential light chain (P53014) Evalue: 5e-31	385	28762	8 (6)	32,8	7,55
F89	contig08542:152:670:2 similar to Probable myosin regulatory light chain (Q09510) Evalue: 4e-50	37	18848	1	6,4	4,96
F93	contig16928:127:297:1 similar to 60S acidic ribosomal protein P1 (O01359) Evalue: 2e-19	35	6118	1	28,1	4,2
F94	contig26443:1:303:1 No Annotation	47	11655	1	12,9	4,72
	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
	contig28231:1:506:3 No Annotation	37	19585	1	6	4,99
	contig18537:1:1312:2 No Annotation	33	49479	1	2,1	5,84
F95	contig27950:148:1366:1 similar to Probable V-type proton ATPase subunit B (Q19626) Evalue: 0.0	103	45013	2	6,9	5,39
	contig18537:1:1312:2 No Annotation	81	49479	2	5,7	5,84
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	66	36658	2	6,6	5,84
	contig28231:1:506:3 No Annotation	51	19585	1	6	4,99
	contig13035:1:170:1 No Annotation	40	6468	1	14,3	5,25
	contig19083:167:1503:2 similar to Low-density lipoprotein receptor (P35951) Evalue: 7e-05	38	51426	1	2,2	5,32
	contig08625:1:110:2 No Annotation	37	4172	1	22,2	9,05
	contig19345:270:876:3 No Annotation	29	23208	1	4,5	5,13
	contig26443:1:303:1 No Annotation	29	11655	1	10,9	4,72
	contig24106:1:138:1 similar to Probable serine carboxypeptidase CPVL (Q9H3G5) Evalue: 5e-09	28	5363	1	28,3	9,69
F96	contig06721:1:451:2 No Annotation	33	16943	1	10,7	4,73

## 7.2 Supplementary II

**Identification and semi-quantitative analysis of proteins from *M. tardigradum* in three different states.** The whole protein lysate of tardigrades in early embryonic state (EES), adults in active state (AS) and tun state (TS) were analyzed utilizing 1D gel electrophoresis in combination with high sensitive nanoLC ESI-MS/MS on a LTQ-Orbitrap mass spectrometer. Generated MS/MS data were searched against the 454 tardigrade protein database. Identified proteins with annotation are listed. Protein annotation, total protein score, number of matched peptides, sequence coverage protein length and emPAI are listed. The calculated emPAI can be used to compare the expression level of each protein in different states.

Protein annotation	Protein score	Peptide matches (unique pept)	Protein coverage	Protein length	emPAI		
					EES	AS	TS
<b>Chaperones</b>							
<b>Hsp90 family</b>							
contig26899:1:722:2 similar to Heat shock protein HSP 90-alpha (P07900) Evalue: 6e-93	105	2	10.4	240		0.26	0.26
contig25094:2604:4781:3 similar to Heat shock protein HSP 90-alpha (Q76LV2) Evalue: 0.0	2690	108 (45)	62.3	725	21.33	19.82	13.85
contig20254:1:1418:3 similar to Heat shock protein 81-2 (Q69QQ6) Evalue: 8e-163	270	9 (6)	13	471			0.32
contig00854:1:1427:3 similar to Endoplasmic (Q95M18) Evalue: 1e-143	484	12 (11)	25.5	474	1.65	3.04	2.56
contig02760:1:1133:2 similar to Endoplasmic (P08113) Evalue: 2e-101	488	10 (9)	29.4	377	0.59	2.67	1.37
<b>Hsp70 family</b>							
contig03976:126:2186:3 similar to Chaperone protein dnaK (A6T4F4) Evalue: 0.0	2128	62 (36)	54.5	686	4.36	8.48	8.05
contig26153:1:1449:2 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	1512	67 (24)	49.4	482	5.12	12.97	9.62
contig03529:113:1972:2 similar to 78 kDa glucose-regulated protein (Q24798) Evalue: 0.0	327	9 (5)	12.3	619	0.1	0.37	0.63
contig02386:1:407:3 similar to 78 kDa glucose-regulated protein (Q90593) Evalue: 9e-42	526	15 (7)	58.2	134	3.23	9.72	11.62
contig24686:114:732:3 similar to Heat shock 70 kDa protein 4 (P11145) Evalue: 2e-90	325	8 (4)	25.2	206	0.99	1.5	2
contig24452:1:1324:1 similar to Heat shock cognate 70 kDa protein 2 (P27322) Evalue: 7e-146	116	3	9.1	441		0.29	0.63
contig15471:1:392:1 similar to Heat shock 70 kDa protein A (P09446) Evalue: 2e-54	178	13 (4)	22.3	130		1.15	0.91
contig27023:167:1862:2 similar to Heat shock protein 105 kDa (Q5R606) Evalue: 2e-153	1204	26 (19)	50.8	565	2.74	2.47	2.97
contig23865:1:793:3 similar to Hypoxia up-regulated protein 1 (Q9Y4L1) Evalue: 2e-24	65	2	9.9	263			0.24
contig11113:142:505:1 similar to Hypoxia up-regulated protein 1 (Q5ZLK7) Evalue: 8e-15	57	1	9.1	121		0.5	
contig01386:1:662:2 similar to Hypoxia up-regulated protein 1 (Q0VA61) Evalue: 1e-55	191	5	34.1	220	0.29	1.04	0.89
contig12271:1:402:2 similar to Heat shock cognate 71 kDa protein (P19120) Evalue: 2e-47	388	13 (6)	58.6	133	7.85	10.32	5.73
contig09502:237:2105:3 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	1374	38 (19)	38.1	622	1.97	3.64	3.66
contig07915:1:1267:2 similar to Heat shock cognate 71 kDa protein (Q90473) Evalue: 0.0	1653	79 (26)	64.2	422	30.43	32.48	21.57
contig07914:1:483:1 similar to Heat shock 70 kDa protein cognate 4 (Q9U639) Evalue: 9e-73	381	16 (8)	43.1	160	2.27	5.82	4.42
contig06011:1:452:3 similar to Heat shock 70 kDa protein 4 (P34932) Evalue: 1e-15	518	15 (9)	57.7	149	3.06	4.03	3.84
<b>Hsp60 family</b>							
contig24176:1:143:1 similar to 60 kDa heat shock protein, mitochondrial (Q5ZL72) Evalue: 4e-10	147	1	53.2	47	9.78	10.27	11.04
contig07413:1:1541:1 similar to 60 kDa heat shock protein, mitochondrial (P63038) Evalue: 0.0	1985	77 (32)	66.1	513	10.73	13.95	12.12
<b>DnaJ (Hsp40) family</b>							
contig22705:72:1160:3 similar to DnaJ homolog subfamily C member 9 (Q91WN1) Evalue: 2e-34	95	2	6.9	362	0.17		
contig20236:3408:4481:3 similar to DnaJ homolog subfamily B member 11 (Q5RAJ6) Evalue: 5e-112	134	2	7.8	357		0.17	0.25
contig17613:1:1068:3 similar to DnaJ homolog subfamily C member 21 (Q6PGY5) Evalue: 3e-05	37	2 (1)	3.1	355	0.24		0.16
contig11009:1:950:1 similar to DnaJ homolog subfamily C member 13 (O75165) Evalue: 4e-84	77	2	6.6	316			0.19
contig07692:388:2166:1 similar to DnaJ homolog subfamily C member 3 (Q5Z113) Evalue: 4e-87	61	2	3	592			0.1
contig06040:2099:3274:1 similar to DnaJ homolog subfamily A member 2 (O60884) Evalue: 3e-86	417	9	33.5	391	1.07	0.93	1.07
contig01842:1:1128:1 similar to DnaJ homolog subfamily A member 1 (Q5E954) Evalue: 3e-97	143	3	10.4	375	0.68	0.5	
contig01190:1:651:1 similar to DnaJ homolog subfamily B member 1 (P25685) Evalue: 2e-62	100	3	17.1	216		0.48	0.3

<b>GrpE family</b>								
contig17853:205:969:1 similar to GrpE protein homolog 1, mitochondrial (Q5RA81) Evalue: 1e-47	305	7 (6)	28.7	254	1.06	1.06	1.06	
<b>sHsp (Hsp 20) family</b>								
contig04304:1:362:3 similar to Small heat shock protein C4 (Q4UJB1) Evalue: 1e-17	63	2	19.3	119	0.56			
contig01972:1:390:1 similar to Protein lethal(2)essential for life (P82147) Evalue: 1e-12	183	8 (3)	30	130	1.02	2.53	3.5	
contig20502:1:416:2 similar to Major egg antigen (P12812) Evalue: 1e-05	71	2	18.8	138	0.45			
contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	793	52 (12)	75.3	166	227.19	108.34	97.24	
contig11265:1:246:1 similar to Heat shock protein beta-1 (P42930) Evalue: 5e-12	77	1	24.7	81		0.74	0.74	
contig01971:1:38:399:3  AGAP000941-PA [Anopheles gambiae str (XP_560153.3)] Evalue: 8e-05	425	17 (7)	58.6	87	2.37	30.05	16.78	
<b>GroES chaperonin family</b>								
contig08799:2167:2550:3 similar to 10 kDa heat shock protein, mitochondrial (Q5DC69) Evalue: 1e-34	202	7 (5)	40.2	127	2.53			
<b>TCP-1 chaperonin family</b>								
contig18669:1:1620:1 similar to T-complex protein 1 subunit eta (Q5R5C8) Evalue: 0.0	1175	24 (21)	53.4	539	4.18	3.45	3.46	
contig04755:1:285:1 similar to T-complex protein 1 subunit alpha (P12613) Evalue: 4e-31	59	2	20.2	94	0.77	1.1	0.77	
contig04754:1:1443:1 similar to T-complex protein 1 subunit alpha A (P11984) Evalue: 0.0	736	15 (12)	32.2	481	2.84	1.9	2.47	
contig26285:1:350:2 similar to T-complex protein 1 subunit gamma (P80318) Evalue: 2e-37	200	5 (3)	35.3	116	0.54	2.28	1.04	
contig24467:1:1384:2 similar to T-complex protein 1 subunit gamma (P48605) Evalue: 0.0	984	22 (16)	43.7	460	2.67	2.26	2.71	
contig24462:119:1723:2 similar to T-complex protein 1 subunit beta (P78371) Evalue: 0.0	781	13 (11)	28.3	534	3.69	2.32	2.6	
contig22788:1:784:2 similar to T-complex protein 1 subunit epsilon (P48643) Evalue: 6e-116	535	14 (11)	54.6	260	2.53	3.81	3.81	
contig21263:1:1713:1 similar to T-complex protein 1 subunit delta (P53451) Evalue: 0.0	634	18 (13)	35.4	570	2.26	1.45	1.64	
contig20686:1:1600:2 similar to T-complex protein 1 subunit theta (P42932) Evalue: 0.0	741	17 (15)	32.6	533	2.88	3.21	2.72	
contig19267:1:1403:3 similar to T-complex protein 1 subunit zeta (P80317) Evalue: 1e-161	590	13 (12)	30.5	466	2.07	2.11	2.4	
contig19251:1970:2808:2 similar to T-complex protein 1 subunit epsilon (P80316) Evalue: 5e-109	580	10 (8)	44.8	279	2.91	2.69	3.09	
<b>Calreticulin family</b>								
contig17826:1:1166:3 similar to Calnexin (P24643) Evalue: 5e-62	202	5 (4)	13.7	388	0.63	0.6	0.24	
contig16173:1:541:3 similar to Calnexin (P27824) Evalue: 3e-11	60	2	15.6	179	0.36	0.36		
contig18504:154:1359:1 similar to Calreticulin (Q4R6K8) Evalue: 4e-127	1370	63 (24)	73.6	401	10.14	19.56	17.54	
<b>AHA1 family</b>								
contig03076:1:682:1 similar to Activator of 90 kDa heat shock protein ATPase homolog 1 (O95433) Evalue: 2e-30	84	3	9.7	227	0.27			
<b>Antioxidant proteins</b>								
<b>Cu-Zn superoxide dismutase family</b>								
contig17128:1:666:1 similar to Superoxide dismutase [Cu-Zn] (Q8HXQ3) Evalue: 5e-52	512	25 (7)	37.1	221	10.94	7.3	5.72	
contig10844:1:307:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 7e-25	203	4	65.3	101	29.04	6.87	15.04	
contig07268:293:706:2 similar to Superoxide dismutase [Cu-Zn] (Q8HXQ2) Evalue: 2e-07	30	1	5.1	138	3.15	0.44	0.44	
contig06938:1:111:1 similar to Superoxide dismutase [Cu-Zn] (O46412) Evalue: 2e-08	74	2	70.3	37	24.66	7.12	5.3	
contig00081:1:487:2 similar to Superoxide dismutase [Cu-Zn] (O73872) Evalue: 1e-42	273	13 (4)	28	161	3.68	4.38	3.88	
contig27339:102:582:3 similar to Superoxide dismutase [Cu-Zn], chloroplastic (P07505) Evalue: 4e-22	92	2	13.8	160			0.83	
contig25087:1:383:2 similar to Copper chaperone for superoxide dismutase (O14618) Evalue: 5e-29	85	2	20.5	127	0.56	0.56	0.5	
<b>iron/manganese superoxide dismutase family</b>								
contig24565:1:222:1 similar to Superoxide dismutase [Mn], mitochondrial (P09671) Evalue: 3e-21	47	2 (1)	13.7	73		0.8		
contig21632:1:563:1 similar to Superoxide dismutase [Mn], mitochondrial (P09671) Evalue: 3e-44	567	16 (9)	50.8	187	3.68	4.95	7.48	

**GST superfamily**

GST superfamily. Mu family.

contig05084:1:468:1 similar to Glutathione S-transferase (O18598) Evalue: 2e-11	121	4 (3)	17.4	155		0.97	
contig04771:2479:3345:1 similar to Glutathione S-transferase (P46419) Evalue: 1e-31	328	7 (6)	25.3	288	0.55	0.74	1.1
contig25262:1:948:1 similar to Glutathione S-transferase (P46428) Evalue: 1e-19	66	2 (1)	5.1	315			0.18
contig24978:1:466:2 similar to Glutathione S-transferase (O18598) Evalue: 4e-13	147	4	18.2	154		1.17	1.35
contig18624:208:876:1 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-34	299	8 (6)	36	222		1.95	1.3
contig18536:108:511:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-25	164	3 (2)	18.7	134		0.79	1.65
contig11340:1490:2110:2 similar to Glutathione S-transferase 1 (P46436) Evalue: 1e-30	137	2	13.6	206		0.31	0.49
contig11153:105:404:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 2e-22	443	17 (9)	76	100	0.68	16.9	9.43
contig21746:1:589:2 similar to Glutathione S-transferase 1 (P46436) Evalue: 6e-32	716	42 (11)	60	195	6.63	19.22	21.28
contig21268:161:805:2 similar to Glutathione S-transferase 1 (P46434) Evalue: 1e-23	212	3	18.2	214		0.47	0.47
contig19542:93:722:3 similar to Glutathione S-transferase 1 (P46436) Evalue: 1e-31	305	10 (5)	27.3	209	0.69	2.03	1.52
contig19224:1:520:3 similar to Glutathione S-transferase 2 (P20136) Evalue: 4e-30	184	5 (4)	32	172		0.83	0.57
contig19966:1:524:3 similar to Microsomal glutathione S-transferase 2 (Q2KJG4) Evalue: 2e-17	33	1	5.2	173	3.03	0.51	1
contig27884:1:341:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-10	224	10 (4)	31	113	2.06	2.6	1.03
contig27967:1:110:3 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-04	129	5 (3)	55.6	36	2.37	6.02	2.37
contig28284:1:272:3 similar to Glutathione S-transferase (P30116) Evalue: 2e-10	361	15 (8)	76.4	89	2.83	16.67	9.79
contig28200:1:335:1 similar to Glutathione S-transferase Mu 1 (Q9N0V4) Evalue: 4e-19	256	11 (6)	32.4	111	3.67	5.42	2.22
contig22559:1:111:1 similar to Glutathione S-transferase Mu 2 (Q9TSM4) Evalue: 1e-05	194	12 (4)	81.1	37	10.36	20.7	12.73
contig28262:1:137:2 similar to Glutathione S-transferase Mu 2 (P08010) Evalue: 2e-04	120	9 (2)	71.1	45	2.45	15.71	23
contig11431:61:646:1 similar to Glutathione S-transferase omega-1 (P78417) Evalue: 4e-24	393	11 (7)	60	195	0.32	2.7	1.02
contig03609:1:822:1 similar to Glutathione S-transferase theta-1 (P20135) Evalue: 2e-28	315	7	29.7	273	0.6	1.13	0.82
contig27735:1:141:1 similar to Probable glutathione transferase omega-2 (P34277) Evalue: 2e-04	73	2	39.1	46		1.83	7.03
contig27010:1:263:3 similar to Glutathione S-transferase class-mu 26 kDa isozyme 7 (P31671) Evalue: 2e-08	197	4 (3)	26.7	86		1.42	
contig27010:1:263:3 similar to Glutathione S-transferase class-mu 26 kDa isozyme 7 (P31671) Evalue: 2e-08	163	4 (3)	26.7	86			1.42
contig26098:1:627:1 similar to Glutathione S-transferase omega-1 (P78417) Evalue: 6e-39	702	23 (13)	68.9	209	6.53	9.78	11.18
contig25826:1:785:3 similar to Glutathione S-transferase omega-1 (Q9N1F5) Evalue: 2e-29	317	8 (6)	31.8	261		0.88	0.52
contig23837:230:385:2 similar to Probable glutathione S-transferase 7 (P91253) Evalue: 7e-08	111	4 (2)	40.4	52		2.71	3.6
contig22893:435:602:3 similar to Glutathione S-transferase (P46428) Evalue: 6e-07	60	2 (1)	23.2	56		1.08	1.08

**GST superfamily. sigma family.**

contig09885:332:509:2 similar to Glutathione-requiring prostaglandin D synthase (O60760) Evalue: 2e-12	117	4 (2)	39	59	1.06	2.66	3.09
contig02240:94:701:1 similar to Glutathione-requiring prostaglandin D synthase (O73888) Evalue: 2e-13	283	18 (4)	23.3	202	0.96	2.51	3.95
contig26589:66:218:3 similar to Glutathione-requiring prostaglandin D synthase (Q9JHF7) Evalue: 6e-11	91	5 (2)	31.4	51		1.49	4.42
contig18259:2282:2926:2 similar to Glutathione-requiring prostaglandin D synthase (O60760) Evalue: 8e-31	321	8 (6)	42.5	214	1.52	2.63	3.18

**GST superfamily. Zeta family.**

contig25932:359:1012:2 similar to Maleylacetoacetate isomerase (Q9WVLO) Evalue: 4e-65	367	8 (6)	40.6	217		1.51	1.1
contig02881:144:845:3 similar to Maleylacetoacetate isomerase (Q9WVLO) Evalue: 2e-56	189	4 (3)	28.8	233		0.55	0.27

**MAPEG family****thioredoxin family**

contig19966:1:524:3 similar to Microsomal glutathione S-transferase 2 (Q2KJG4) Evalue: 2e-17	33	1	5.2	173	3.03	0.51	1
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contig03216:160:412:1 similar to Thioredoxin-1 (O14463) Evalue: 2e-20	205	5 (4)	56	84	4.5	3.27	3.27
contig27083:1:464:3 similar to Thioredoxin-like protein 1 (O43396) Evalue: 8e-34	160	5 (4)	41.6	154	1.08	1.08	0.88
contig19284:1:135:1 similar to Thioredoxin-like protein 1 (O43396) Evalue: 2e-08	73	1	45.5	44		2.91	1.54
contig22753:96:617:3 similar to Thioredoxin, mitochondrial (Q95108) Evalue: 2e-37	31	1	6.9	173	1.83	0.9	0.72
contig01577:1:665:3 similar to Thioredoxin domain-containing protein 9 (Q9CQ79) Evalue: 4e-48	167	3	15.9	220	0.92	0.45	
<b>glutaredoxin family</b>							
contig18103:93:590:3 similar to Glutaredoxin-related protein 5 (Q80Y14) Evalue: 3e-36	123	2	13.9	165	0.8		
contig18548:126:1097:3 similar to Glutaredoxin-3 (Q28ID3) Evalue: 5e-82	82	2	7.1	323	0.53	0.19	0.29
<b>ahpC/TSA family</b>							
contig24044:157:536:1 similar to Peroxiredoxin-2 (Q2PFZ3) Evalue: 2e-33	270	9 (4)	28.6	126	4.02	3.19	4.62
contig23916:1:215:3 similar to Peroxiredoxin-2 (Q8K3U7) Evalue: 7e-30	96	4 (2)	25.7	70		3.12	3.12
contig21646:1:212:3 similar to Peroxiredoxin-2 (Q8K3U7) Evalue: 7e-29	187	6 (3)	75.4	69	3.63	7.06	3.92
contig17026:72:905:3 similar to Peroxiredoxin-2 (Q2PFZ3) Evalue: 5e-75	215	8 (4)	17.3	277	0.59	1.02	1.2
contig24578:1:638:1 similar to Peroxiredoxin-4 (O08807) Evalue: 8e-51	313	8 (7)	42	212	2.32	2.82	2.25
contig20474:30:605:3 similar to Peroxiredoxin-5, mitochondrial (P30044) Evalue: 2e-48	198	6 (4)	24.6	191	0.9	1.15	0.72
contig18110:116:595:2 similar to Peroxiredoxin-5, mitochondrial (Q9GLW9) Evalue: 2e-44	94	2	16.4	159	0.42		
contig00858:452:928:2 similar to Peroxiredoxin-6 (Q5ZJF4) Evalue: 3e-45	445	16 (8)	63.9	158	2.26	6.96	7.82
contig08182:1:405:1 similar to Thioredoxin-dependent peroxide reductase, mitochondrial (P20108) Evalue: 7e-47	70	2	19.4	134		0.5	0.73
<b>glutathione peroxidase family</b>							
contig17497:1:693:1 similar to Glutathione peroxidase (Q00277) Evalue: 5e-45	403	16 (8)	42.2	230	1.76	2.46	2.33
<b>class-I pyridine nucleotide-disulfide oxidoreductase family</b>							
contig28039:246:1925:3 similar to Thioredoxin reductase 1, cytoplasmic (Q16881) Evalue: 1e-165	641	15 (12)	34.3	559	0.34	1.08	0.73
<b>Oxidoreductases</b>							
<b>peroxidase family. XPO subfamily.</b>							
contig25378:1:753:2 similar to Chorion peroxidase (P82600) Evalue: 4e-35	70	2	7.2	250		0.37	0.12
contig25378:1:753:2 similar to Chorion peroxidase (P82600) Evalue: 4e-35	41	1	2.8	250			
contig04779:1:1782:1 similar to Chorion peroxidase (Q9VEG6) Evalue: 6e-75	88	2	3.9	593		0.1	0.1
contig13216:1:1478:3 similar to Peroxidasin homolog (Q3UQ28) Evalue: 7e-114	45	2 (1)	2.4	491	0.3		
contig11268:1:905:3 similar to Peroxidasin (Q9VZZ4) Evalue: 6e-41	376	6 (5)	26.7	300	0.3	0.85	0.45
contig09780:655:1395:1 similar to Peroxidasin homolog (Q92626) Evalue: 2e-14	338	7 (6)	30.4	247		1.25	1.01
contig07623:1:1128:1 similar to Peroxidasin homolog (Q3UQ28) Evalue: 6e-59	381	6	19.9	376		0.56	0.56
contig22552:1:2159:2 similar to Peroxidasin (A4IGL7) Evalue: 7e-54	1462	47 (24)	55.8	719		3.79	2.75
<b>zinc-containing alcohol dehydrogenase family. Quinone oxidoreductase subfamily.</b>							
contig27412:1:469:2 similar to Quinone oxidoreductase (P47199) Evalue: 1e-15	163	4 (3)	23.2	155		1.27	0.7
contig00766:103:1068:1 similar to Quinone oxidoreductase (P47199) Evalue: 6e-76	323	8 (6)	23.4	321		0.93	0.64
contig18169:1:682:3 similar to Quinone oxidoreductase (Q5R4S7) Evalue: 3e-44	99	3	25.2	226			0.3
contig25029:52:537:1 similar to Quinone oxidoreductase (Q6AYT0) Evalue: 2e-38	109	2	19.8	162			0.42
contig17906:1:693:3 similar to Quinone oxidoreductase (Q6AYT0) Evalue: 4e-30	369	6 (5)	34.3	230		1.31	1.02
<b>ferredoxin--NADP reductase type 1 family</b>							
contig26225:1:1238:3 similar to NADPH:adrenodoxin oxidoreductase, mitochondrial (P82861) Evalue: 2e-104	62	2	4.6	411		0.15	0.23



<b>short-chain dehydrogenases/reductases (SDR) family</b>								
contig23428:1:781:2 similar to Uncharacterized oxidoreductase yxC (P40397) Evalue: 2e-59	508	17 (9)	38.2	259	2.14			1.54
contig25297:1:781:2 similar to Uncharacterized oxidoreductase yxC (P40397) Evalue: 2e-58	511	14 (9)	45.2	259	9.06	2.41		2.02
contig24685:241:1143:1 similar to Uncharacterized oxidoreductase yxC (P40397) Evalue: 2e-71	235	5	18.3	300	2.73	0.72		0.66
contig18534:1992:2753:1 similar to Uncharacterized oxidoreductase yxC (P40397) Evalue: 3e-61	74	2	7.5	253	0.25	0.25		0.25
contig00386:1:827:3 similar to Uncharacterized oxidoreductase yhdF (O07575) Evalue: 1e-74	232	7 (6)	34.3	274		1.63		1.55
contig21076:1:608:3 similar to Uncharacterized oxidoreductase yhdF (O07575) Evalue: 6e-58	73	1	5.5	201	1.71	0.3		
contig20766:1:786:1 similar to Uncharacterized oxidoreductase yhdF (O07575) Evalue: 1e-49	523	11 (8)	39.5	261	1.22	3.85		3.76
contig09201:123:466:3 similar to Uncharacterized oxidoreductase yhdF (O07575) Evalue: 9e-18	84	2	19.3	114	2.83	1.2		1.62
contig05892:363:620:3 similar to Uncharacterized oxidoreductase yhdF (O07575) Evalue: 2e-10	164	3 (2)	32.6	86	1.14	1.29		1.67
contig06705:145:450:1 similar to Uncharacterized oxidoreductase TM_0325 (Q9WYGO) Evalue: 3e-08	96	3 (2)	37.3	102	1.32			0.64
contig00126:38:435:2 similar to Probable oxidoreductase ephD (P66778) Evalue: 9e-11	37	1	7.6	132		0.72		
<b>aldo/keto reductase family</b>								
<b>complex I 75 kDa subunit family</b>								
contig22965:1:959:3 similar to Uncharacterized oxidoreductase ZK1290.5 (Q09632) Evalue: 1e-65	158	5 (3)	17.3	318	0.28	0.6		0.28
contig11701:1:1448:1 similar to NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial (P15690) Evalue: 9e-169	678	12 (11)	36.1	482	0.2	1.36		1.17
contig21597:124:783:1 similar to NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial (Q94511) Evalue: 4e-96	103	4 (3)	11.8	220		0.67		0.61
<b>complex I 51 kDa subunit family.</b>								
contig09616:1:1244:3 similar to NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial (Q91YT0) Evalue: 0.0	228	5	16.5	413	0.15	0.48		0.52
contig21698:72:454:3 similar to NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial (Q0MQI8) Evalue: 2e-37	145	4 (3)	30.7	127		0.88		0.88
contig02969:1:262:2 similar to NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial (P19234) Evalue: 4e-22	75	2	43	86		0.86		
<b>FAD-dependent oxidoreductase 2 family. FRD/SDH subfamily.</b>								
contig02931:68:2026:2 similar to Probable succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (Q9UTJ7) Evalue: 0.0	1152	27 (18)	37.4	652	0.61	2.51		1.64
<b>ETF alpha-subunit/fixB family.</b>								
contig20530:1:997:2 similar to Electron transfer flavoprotein subunit alpha, mitochondrial (Q8HXY0) Evalue: 3e-107	867	20 (13)	53.8	331	2.93	3.64		3.5
contig17994:1:644:2 similar to Electron transfer flavoprotein subunit beta (P38117) Evalue: 8e-67	428	11 (9)	59.8	214	1.36	3.92		3.06
<b>intradial ring-cleavage dioxygenase family.</b>								
contig26540:1:668:3 similar to Chlorocatechol 1,2-dioxygenase (P27098) Evalue: 7e-14	185	5 (3)	19	221	0.29	0.88		1.01
<b>aldo/keto reductase family.</b>								
contig08420:165:1178:3 similar to Prostaglandin F synthase 1 (P05980) Evalue: 2e-58	48	1	2.1	337				0.16
<b>catalase family. HP11 subfamily</b>								
contig01298:1:2065:3 similar to Catalase HP11 (P21179) Evalue: 0.0	1706	67 (29)	55	687	7.24	13.23		11.24
<b>globin family. Two-domain flavohemoproteins subfamily.</b>								
contig21229:1:290:3 similar to Flavohemoprotein (Q8ETH0) Evalue: 7e-04	167	2	31.6	95	0.73	1.77		2
<b>others</b>								
contig01508:1:845:3  putative dioxygenase [Pseudomonas fluorescens SBW25] (YP_002872512.1) Evalue: 6e-35	143	3	12.8	281	0.21	0.43		0.21
contig18735:1:831:1 similar to Oxidoreductase HTATIP2 (Q9Z2G9) Evalue: 1e-51	124	3	11.6	276	0.22	0.46		0.33
contig20134:1:1502:2 similar to Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial (P55931) Evalue: 0.0	200	5	15.6	500	0.19	0.32		0.19

<b>Inhibitors</b>							
contig22765:1:560:3 similar to CD109 antigen (Q6YHK3) Evalue: 3e-16	422	12 (8)	40	185	1.54	17.92	15.09
contig18156:1490:2302:3 similar to CD109 antigen (Q6YHK3) Evalue: 6e-36	151	5	13	270			0.78
contig16862:1:302:1 similar to CD109 antigen (Q6YHK3) Evalue: 8e-07	80	1	12	100		1.2	1
contig14704:1:1125:1 similar to CD109 antigen (Q8R422) Evalue: 1e-38	215	5	14.2	374		0.86	0.82
contig14499:601:864:1 similar to CD109 antigen (Q6YHK3) Evalue: 1e-14	166	5 (2)	36.4	88		7.98	9.81
contig01519:1:1386:1 similar to CD109 antigen (Q8R422) Evalue: 2e-80	909	18 (14)	40	462	0.53	8.41	8.04
contig23619:142:1410:1 similar to Alpha-2-macroglobulin receptor-associated protein (Q99068) Evalue: 2e-12	527	12 (11)	31.3	422	8.26	2.05	3.35
contig14499:1:464:3 similar to Alpha-2-macroglobulin-like protein 1 (A8K2U0) Evalue: 9e-05	224	4	24.8	153	0.61	6.41	5.97
contig09859:1:353:3 similar to Ovostatin (P20740) Evalue: 2e-19	100	1	12.9	116		1.08	0.81
contig15368:1:317:2 similar to Murinoglobulin-1 (P28665) Evalue: 4e-17	40	1	6.7	105		0.56	
<b>Serpin family (Serine protease inhibitor)</b>							
contig09540:1:494:2 similar to Plasminogen activator inhibitor 1 (P50449) Evalue: 1e-04	73	2	17.1	164			0.4
contig19800:1:1133:3 similar to Leukocyte elastase inhibitor A (Q9D154) Evalue: 7e-57	919	23 (15)	42.8	376	1.03	7.09	6.43
contig00038:1:298:2 similar to Leukocyte elastase inhibitor A (Q9D154) Evalue: 3e-10	32	1	7.1	98	0.64	0.32	0.64
contig24774:1:1265:3 similar to Serpin B4 (P48594) Evalue: 2e-49	211	3	11.2	420		0.22	0.6
contig03064:1:1299:1 similar to Serpin B8 (P50452) Evalue: 2e-53	418	14 (8)	24.5	432		1.05	0.92
contig28141:1:1138:2 similar to Serpin B10 (B3RFC3) Evalue: 8e-45	511	13 (9)	34.1	378		1.52	0.95
contig25144:101:1438:2 similar to Serpin B10 (B3RFC3) Evalue: 2e-43	176	4	9.2	445	0.37		0.3
contig24933:131:844:2 similar to Serpin B3 (P29508) Evalue: 6e-21	141	3	16	237	0.56		
contig22116:1:603:1 similar to Serpin I2 (Q9JK88) Evalue: 1e-18	293	5	32	200	0.77	1.37	0.77
contig19308:1:1054:3 similar to Serpin B3 (P29508) Evalue: 2e-45	1107	37 (15)	59.1	350	1.42	6.98	6.8
contig01833:1:1065:1 similar to Serpin B3 (P29508) Evalue: 2e-40	525	11 (10)	34.5	354	0.08	2.25	2.25
contig19095:1:1026:1 similar to Serpin B5 (Q6GLQ1) Evalue: 3e-18	705	15 (12)	51.3	341	0.17	2.26	2.23
contig18156:143:1384:2 similar to Serpin I2 (Q9JK88) Evalue: 1e-35	179	3	9.2	413	0.23		
contig17915:352:1599:1 similar to Serpin B3 (P29508) Evalue: 3e-33	42	1	2.2	415	0.14		
contig14730:1:321:1 similar to Uncharacterized serpin-like protein MA_2246 (Q8TNN7) Evalue: 1e-04	99	2	21.7	106	0.91		
contig17490:79:1254:1 similar to Uncharacterized serpin-like protein MM_2675 (Q8PTN8) Evalue: 1e-34	929	28 (19)	48.3	391	0.23	5.8	3.36
<b>Papilin family (Serine protease inhibitor)</b>							
contig07766:1:563:3 similar to Papilin (O95428) Evalue: 4e-18	161	7 (3)	22	186	1.94	1.37	1.5
contig01516:1:634:2 similar to Papilin (Q868Z9) Evalue: 2e-19	88	2	10.4	211	1.47	0.61	1.06
<b>Cystatin family (Thiol proteinase inhibitor)</b>							
contig27581:79:438:1 similar to Cystatin-B (Q862Z5) Evalue: 2e-13	207	15 (5)	42	119	8.69		0.54
contig17172:816:1172:2 similar to Cystatin-B (Q8I030) Evalue: 7e-17	140	4 (3)	28	118	2.2		
<b>Others</b>							
contig18104:168:770:3 similar to Serine protease inhibitor dipetalogastin (O96790) Evalue: 1e-14	393	15 (9)	49	200	0.45	6.03	5.02
<b>ATPase inhibitor family</b>							
contig26952:1:404:3 similar to ATPase inhibitor mai-2, mitochondrial (A8XZB0) Evalue: 1e-09	90	2	17.2	134		0.72	2.07
<b>Proteasome inhibitor PI31 family</b>							
contig24126:143:870:2 similar to Putative proteasome inhibitor (Q9V637) Evalue: 3e-09	126	2	17.8	242	0.24	0.38	0.5

<b>CRISP family</b>								
contig08352:51:734:3 similar to Ophanin (Q7ZT98) Evalue: 1e-24	485	19 (9)	49.3	227	0.42	5.64	4.4	
<b>Peptidases</b>								
<b>peptidase S1 family. Plasminogen subfamily.</b>								
contig23591:1:328:2 similar to Plasminogen (P06868) Evalue: 4e-11	136	3	46.3	108	1.81	1.85	1.85	
<b>seine protease</b>								
contig23591:1:328:2 similar to Plasminogen (P06868) Evalue: 4e-11	240	5 (4)	46.3	108				
contig03269:1:1861:3 seems to belong to peptidase S1 family Best-hit: Plasminogen (Q01177) Evalue: 3e-36	227	4	9.9	619		0.25	0.15	
<b>peptidase S1 family. Plasma kallikrein subfamily.</b>								
contig05316:295:1869:1 similar to Plasma kallikrein (Q2KJ63) Evalue: 4e-19	105	2	4.8	524	0.11	0.17	0.17	
contig04234:1:1412:1 similar to Plasma kallikrein (P26262) Evalue: 5e-24	87	2	5.3	470	0.19	0.19	0.12	
contig24422:1:599:3 similar to Plasma kallikrein (P26262) Evalue: 1e-12	255	6 (4)	38.9	198		0.92	1.5	
<b>Peptidase A1 family (aspartyl proteases)</b>								
contig17912:1:115:2 similar to Lysosomal aspartic protease (Q03168) Evalue: 1e-04	66	1	29.7	37	2.52			
contig23128:964:1796:1 similar to Lysosomal aspartic protease (Q03168) Evalue: 2e-79	214	5 (4)	26	277		1.43	0.8	
contig21771:1:424:2 similar to Lysosomal aspartic protease (Q03168) Evalue: 3e-48	236	5 (4)	43.6	140		2.6	0.73	
contig20004:1:1020:2 similar to Lysosomal aspartic protease (Q03168) Evalue: 6e-121	350	8 (6)	25.4	339	2.43	1.83	1.73	
<b>Peptidase C1 family (thiol proteases)</b>								
contig08207:1:819:1 similar to Putative cysteine proteinase CG12163 (Q9VN93) Evalue: 8e-83	270	5 (4)	20.2	272	0.44	0.9	1.32	
contig17318:1:1144:2 similar to Cathepsin L (Q26636) Evalue: 1e-33	245	6 (5)	21.8	380		1	1.19	
contig07935:1:901:2 similar to Cathepsin L1 (P06797) Evalue: 2e-25	286	8 (5)	18.1	299		1.38	1.3	
contig06371:1:683:1 similar to Cathepsin L1 (P07154) Evalue: 1e-16	390	10 (9)	41.4	227		3.33	3.94	
contig02046:1:1025:3 similar to Cathepsin L (Q26636) Evalue: 1e-34	62	2 (1)	2.1	340		0.63		
contig00607:21:1139:3 similar to Cathepsin L1 (P06797) Evalue: 5e-39	63	2	4	372			0.16	
contig17881:1:101:3 similar to Cathepsin B (P07688) Evalue: 8e-08	84	2 (1)	53.1	32	4.28	3.21	3.21	
contig17572:1:760:3 similar to Cathepsin L1 (Q9GKL8) Evalue: 1e-59	149	2	11.9	252	2.73	1.05	1.66	
contig18129:136:1026:1 similar to Cathepsin Z (P05689) Evalue: 2e-100	548	13 (9)	37.2	296	3.57	2.16	3.01	
contig18366:49:1152:1 similar to Cathepsin K (O35186) Evalue: 3e-31	864	29 (17)	53.1	367		8.29	5.3	
contig20534:85:1134:1 similar to Cathepsin L1 (Q28944) Evalue: 8e-51	165	3	14	349		0.46	0.27	
contig23569:1:798:1 similar to Cathepsin L2 (O60911) Evalue: 7e-27	578	15 (10)	49.4	265		4.65	4.17	
contig23523:1:350:3 similar to Cathepsin L (Q95029) Evalue: 2e-46	77	2 (1)	12.2	115	1.78	0.54	1.16	
contig22183:1:112:2 similar to Cathepsin B-like cysteine proteinase 6 (P43510) Evalue: 2e-08	36	1	21.6	37		1.82		
contig24357:73:1197:1 similar to Cathepsin L (Q95029) Evalue: 4e-34	257	8 (6)	18.4	374		0.69	0.45	
contig24433:1:1039:2 similar to Cathepsin L1 (Q9GL24) Evalue: 2e-104	306	9 (6)	26.7	345		1.75	1.39	
contig24789:58:1104:1 similar to Cathepsin H (O46427) Evalue: 1e-42	162	4	11.2	348		0.39	0.46	
contig27387:186:553:3 similar to Cathepsin B (P07858) Evalue: 2e-21	99	2	18.9	122	0.77	0.77		
contig27566:1:597:1 similar to Cathepsin L (Q26636) Evalue: 1e-48	139	2	18.7	198	0.81	0.81	1.21	
contig27905:1:454:1 similar to Cathepsin B (P10605) Evalue: 6e-53	180	10 (3)	25.8	151	1.28	4.03	3.04	
contig03308:1:1170:1 similar to Crustapain (Q86GF7) Evalue: 8e-31	209	7 (4)	14.4	389		1.12	0.6	
contig21432:22:1311:1 similar to Crustapain (Q86GF7) Evalue: 7e-34	546	18 (9)	28.9	429		2.32	1.88	
contig27565:1:203:3 seems to belong to peptidase C1 family Best-hit: Cysteine proteinase 2 (Q05094) Evalue: 4e-07	140	4 (3)	47.8	67	4.12			

contig00737:121:1632:1 similar to Uncharacterized peptidase C1-like protein F26E4.3 (P90850) Evalue: 5e-96	377	7	15.5	503	0.39	0.53
<b>peptidase M12A family</b>						
contig11988:1:1216:2 similar to Zinc metalloproteinase nas-14 (Q19269) Evalue: 3e-27	359	6	17.8	404	1.37	0.74
contig03632:189:869:3 similar to Zinc metalloproteinase nas-14 (Q19269) Evalue: 8e-29	104	2	16.4	226		0.27
contig04515:1:928:3 similar to Zinc metalloproteinase nas-4 (P55112) Evalue: 8e-38	368	7 (6)	37	308	0.2	0.89
contig16482:45:1196:3 similar to Zinc metalloproteinase nas-13 (Q20191) Evalue: 1e-31	237	7 (6)	14.6	383		0.65
contig06816:345:1322:3 similar to Zinc metalloproteinase nas-14 (Q19269) Evalue: 3e-45	233	5	20.3	325		0.94
contig06299:302:959:2 similar to Zinc metalloproteinase nas-8 (Q18439) Evalue: 8e-27	120	3	13.7	219		0.7
contig05485:134:1203:2 similar to Zinc metalloproteinase nas-14 (Q19269) Evalue: 4e-39	315	6	16.6	356		0.6
contig02629:233:1093:2 similar to Zinc metalloproteinase nas-14 (Q19269) Evalue: 3e-31	73	2	12.5	287		0.22
contig25998:1:296:3 similar to Embryonic protein UVS.2 (P42664) Evalue: 8e-06	260	14 (6)	59.8	97	5.53	10.17
contig04549:156:1202:3 similar to Embryonic protein UVS.2 (P42664) Evalue: 8e-09	73	1	4.3	348		0.18
<b>peptidase M13 family.</b>						
contig03758:710:1808:2 similar to Neprilysin-1 (Q18673) Evalue: 5e-45	155	3	10.1	366		0.26
contig19059:1:2293:2 similar to Neprilysin-2 (O16796) Evalue: 2e-108	492	11 (10)	16.3	763		0.52
contig19161:1:1557:1 similar to Neprilysin-2 (O16796) Evalue: 2e-87	244	4	12.4	518	0.31	0.48
<b>peptidase M10A family</b>						
contig25188:72:1790:3 similar to Matrix metalloproteinase-16 (P51512) Evalue: 5e-54	411	8	17.5	572	0.25	0.94
<b>Peptidase C56 family</b>						
contig18650:1:487:1 similar to Protein DJ-1 (Q5E946) Evalue: 3e-31	214	5	36.4	162	1.72	1.41
contig27778:718:913:2 similar to Protein DJ-1 (Q5E946) Evalue: 8e-08	245	4 (3)	56.2	64	0.96	2.27
<b>peptidase M1 family</b>						
contig07181:1:2433:1 similar to Puromycin-sensitive aminopeptidase (P55786) Evalue: 0.0	872	18 (16)	29.1	810	1.11	1.58
contig10703:300:2318:3 similar to Aminopeptidase N (P97449) Evalue: 1e-81	218	4 (3)	6	672		0.49
<b>peptidase M2 family.</b>						
contig28013:160:1255:1 similar to Angiotensin-converting enzyme (Q6Q4G4) Evalue: 6e-53	166	3	8.5	365	0.24	0.25
contig25784:1:381:2 similar to Angiotensin-converting enzyme (Q10751) Evalue: 2e-40	77	2	17.5	126		0.53
contig25084:1:1521:2 similar to Angiotensin-converting enzyme (Q50JE5) Evalue: 5e-133	529	18 (10)	26.1	506	1.74	
contig24559:1:1096:3 similar to Angiotensin-converting enzyme (Q50JE5) Evalue: 1e-118	108	2	11	364		0.16
contig05132:1:1108:2 similar to Angiotensin-converting enzyme (P47820) Evalue: 1e-110	74	2	5.7	369	0.16	0.16
contig02953:24:1973:3 similar to Angiotensin-converting enzyme (Q6Q4G4) Evalue: 2e-156	1170	28 (19)	43.8	649		2.88
contig17100:280:1491:1 similar to Peptidase M20 domain-containing protein 2 (Q8IYS1) Evalue: 2e-80	405	11 (10)	32	403	0.24	1.28
contig24518:126:1331:3 similar to Peptidase M20 domain-containing protein 2 (Q8IYS1) Evalue: 2e-83	255	5	16.2	401		0.58
contig00345:128:1143:2 similar to Peptidase M20 domain-containing protein 2 (Q501L1) Evalue: 2e-59	195	4	16	338		0.41
contig03294:1:292:2 similar to Peptidase M20 domain-containing protein 2 (Q501L1) Evalue: 9e-12	36	1	8.3	96		0.66
contig23074:128:1567:2 similar to Probable aminopeptidase NPEPL1 (Q6NSR8) Evalue: 6e-108	527	11 (10)	29.6	480	0.34	1.3
contig20649:1:270:1 similar to Probable aminopeptidase NPEPL1 (Q6NSR8) Evalue: 1e-12	64	2	16.9	89		0.84
contig18449:1:1229:3 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 2e-87	1134	44 (18)	55.4	408	2.6	6.37
contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	708	25 (10)	64	200	3.38	8.95
others						
contig06892:141:2399:3 similar to Glutamate carboxypeptidase 2 (P70627) Evalue: 2e-110	126	2	4.4	752		0.08

contig06137:64:2154:1 similar to Mitochondrial intermediate peptidase (A6H611) Evalue: 3e-169	76	2	3	696		0.08		
contig24645:198:2063:3 similar to Xaa-Pro aminopeptidase 1 (Q1JPJ2) Evalue: 3e-180	218	4	8.7	621		0.3	0.3	
contig06040:1:1489:2 similar to Xaa-Pro dipeptidase (P12955) Evalue: 0.0	248	3	9.7	495		0.38	0.61	
contig00879:1:1167:1 similar to Carboxypeptidase B (P04069) Evalue: 6e-69	127	3	7.2	388		0.25	0.16	
contig06628:84:1388:3 similar to Carboxypeptidase B (P04069) Evalue: 4e-70	451	8	27.4	434		0.82	0.61	
contig19578:1:925:2 similar to PPPDE peptidase domain-containing protein 2 (Q6GLM5) Evalue: 3e-05	281	6 (4)	17.2	308		0.58	0.58	
contig05826:1:988:2 similar to Dipeptidyl peptidase family member 6 (P34422) Evalue: 4e-21	491	11 (9)	32	328	0.41	1.16	1.16	
contig04132:248:522:2 similar to Alpha-aspartyl dipeptidase (Q91642) Evalue: 2e-16	78	3 (2)	28.6	91		0.79		
contig02772:486:1031:3 similar to Signal peptidase complex catalytic subunit SEC11A (Q9R0P6) Evalue: 3e-70	498	10 (8)	64.6	181	1.89			
contig19386:1:890:1 similar to Probable serine carboxypeptidase CPVL (Q9H3G5) Evalue: 2e-74	411	14 (6)	37.2	296	0.52	2.03	1.2	
contig24106:1:138:1 similar to Probable serine carboxypeptidase CPVL (Q9H3G5) Evalue: 5e-09	128	3	71.7	46	1.83	6.49	6.49	
contig26156:52:2316:1 similar to Prolyl endopeptidase (P23687) Evalue: 0.0	693	17 (13)	21.4	754	0.5	0.7	0.71	
contig24450:1109:1479:3 similar to Proline iminopeptidase (P93732) Evalue: 3e-30	199	5	46.7	122		1.95	1.38	
contig18920:1:583:1 similar to Proline iminopeptidase (P52279) Evalue: 1e-70	165	5 (4)	19.6	194		0.74	0.74	
contig17475:1:1628:3 similar to Mitochondrial-processing peptidase subunit beta (Q3SZ71) Evalue: 7e-169	884	35 (14)	41	541	1.45	3.99	2.72	
contig24122:171:1724:3 similar to Mitochondrial-processing peptidase subunit alpha (Q10713) Evalue: 9e-152	145	3	9.3	517	0.18			
contig01139:54:1481:3 similar to Methionine aminopeptidase 2 (P50579) Evalue: 3e-152	172	5	11.6	475	0.35			
contig10192:37:2259:1 similar to Dipeptidyl-peptidase 3 (O55096) Evalue: 7e-180	1317	27 (22)	35	740	0.38	0.59	0.62	
contig04903:63:1787:3 similar to Nicalin (Q969V3) Evalue: 3e-76	250	5	12.7	574		0.28	0.16	
<b>Lipases</b>								
contig24738:99:1589:3 similar to Pancreatic lipase-related protein 2 (P54318) Evalue: 2e-58	127	2	6.5	496			0.12	
contig22166:1:1452:1 similar to Pancreatic lipase-related protein 2 (Q64424) Evalue: 5e-41	158	5 (4)	11.2	483	0.27			
contig22025:104:1296:2 similar to Group XV phospholipase A2 (Q8VEB4) Evalue: 3e-59	122	3	8.3	397	0.24		0.9	
contig20744:89:1051:2 similar to Group 3 secretory phospholipase A2 (Q9NZ20) Evalue: 5e-07	83	2	7.8	320		0.18	0.09	
contig20561:1:604:1 similar to Gastric triacylglycerol lipase (Q9CPP7) Evalue: 7e-53	136	4	20.4	201		0.65		
contig11754:1:2273:3 similar to 85 kDa calcium-independent phospholipase A2 (O60733) Evalue: 3e-121	120	3	6.1	756		0.16		
contig11454:1:1130:3 similar to Hepatic triacylglycerol lipase (P11150) Evalue: 3e-34	409	12 (8)	30.9	375	1.22			
contig10509:753:1454:3 similar to Monoglyceride lipase (O35678) Evalue: 3e-50	95	2	12.8	234		0.27		
contig08576:1:1802:3 similar to Phospholipase A-2-activating protein (Q6GM65) Evalue: 1e-102	356	7	18.5	600	0.41	0.41	0.28	
contig07969:1:1665:1 similar to Phospholipase D3 (Q6PB03) Evalue: 4e-101	48	1	2	554		0.1		
<b>Lipid transport, storage</b>								
<b>Large lipid transporter protein family</b>								
contig26360:1:1109:3 similar to Vitellogenin-2 (P05690) Evalue: 2e-12	1020	35 (19)	57.3	368	25.45	33.27	30.89	
contig26295:1:531:2 similar to Vitellogenin-6 (P18948) Evalue: 4e-16	338	26 (5)	64.2	176	76.29	37.64	40.05	
contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	725	60 (12)	55.5	256	34.26	55.07	60.25	
contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	1321	128 (20)	76.3	317	206.56	107.89	97.94	
contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	1689	126 (24)	67.5	493	107.82	60.6	56.55	
contig02294:1:661:1 similar to Vitellogenin-4 (P18947) Evalue: 5e-11	774	59 (12)	73.2	220	65.94	95.06	73.72	
contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	810	72 (10)	51.4	216	219.67	134.37	113.52	
contig02295:455:757:2 hypothetical protein BRAFLDRAFT_69973 [Branchiostoma floridae] (XP_002591410.1) Evalue: 8e-04	290	32 (5)	52.5	101	72.73	58.29	47.7	
contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	2337	168 (33)	68.6	484	434.48	148.6	131.78	

contig26982:1:1617:2 similar to Microsomal triglyceride transfer protein large subunit (P55158) Evalue: 4e-31	650	16 (13)	22.5	538		1.88	1.93
contig24202:107:1017:2 similar to Apolipoprotein B-100 (P04114) Evalue: 3e-18	914	47 (15)	62	303	106.16	47.92	45.38
contig18537:1:1312:2  PREDICTED: similar to apolipoprotein B [Strongylocentrotus purpuratus] (XP_800206.2) Evalue: 1e-07	1373	99 (21)	51.5	437	141.61	65.75	55.45
<b>LDLR family.</b>							
contig23325:1:1472:3 similar to Very low-density lipoprotein receptor (P35953) Evalue: 4e-24	151	2	9.2	489	1.15	0.12	0.12
contig19083:167:1503:2 similar to Low-density lipoprotein receptor (P35951) Evalue: 7e-05	705	21 (13)	30.3	445	0.36	1.67	1.52
contig04458:347:1819:2 similar to Low-density lipoprotein receptor-related protein 5 (O75197) Evalue: 1e-20	130	2	6.1	490		0.12	0.12
contig25676:1:412:3 similar to Very low-density lipoprotein receptor (P98155) Evalue: 9e-21	54	1	8.1	136		0.42	0.63
contig25250:813:1145:3 similar to Low-density lipoprotein receptor-related protein 2 (A2ARV4) Evalue: 2e-10	157	4 (3)	33.6	110	0.56	2.56	2.38
<b>perilipin family.</b>							
contig18066:109:664:1 similar to Lipid storage droplets surface-binding protein 2 (Q9VXY7) Evalue: 2e-08	265	8 (5)	35.7	185			1.46
contig23310:1:1017:1 similar to Perilipin-2 (Q9TUM6) Evalue: 8e-15	136	3	11.8	338	1.52	0.29	0.29
<b>High density lipoprotein-binding protein</b>							
<b>Vigilin</b>							
contig16622:524:710:2 similar to Vigilin (Q9Z1A6) Evalue: 5e-08	37	1	16.4	61		1.59	1.06
contig08715:1:306:1 similar to Vigilin (Q5R439) Evalue: 8e-21	37	1	7.8	102	0.58	1.16	1.45
contig23870:1:1221:3 similar to Vigilin (Q8VDJ3) Evalue: 1e-76	471	11 (9)	25.4	406	0.49	1.85	1.38
contig20907:1:1151:1 similar to Vigilin (Q8VDJ3) Evalue: 2e-73	409	11 (9)	29.2	383	0.72	2.75	2.05
<b>Ion transport</b>							
<b>X(+)/potassium ATPases subunit beta family.</b>							
contig26273:258:1214:3 similar to Sodium/potassium-transporting ATPase subunit beta (P25169) Evalue: 1e-52	369	14 (7)	24.8	318	0.38	1.99	2.01
contig26215:1:1901:3 similar to Sodium/potassium-transporting ATPase subunit alpha-1 (P04074) Evalue: 0.0	889	22 (14)	24.7	632	0.58	4.94	4.03
contig13779:85:487:1 similar to Sodium/potassium-transporting ATPase subunit alpha (Q6RWA9) Evalue: 2e-33	163	3	38.1	134	0.46	3.18	2.57
contig08413:1:307:1 similar to Sodium/potassium-transporting ATPase subunit alpha (Q6RWA9) Evalue: 4e-44	127	2	25.5	102		1	2.1
<b>cation transport ATPase (P-type) family.</b>							
contig22430:1:860:3 similar to Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type (Q7PPA5) Evalue: 1e-131	469	6 (5)	22.1	285	0.68	5.17	3.75
contig20293:1:217:2 similar to Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type (Q7PPA5) Evalue: 4e-12	212	3 (2)	43.7	71	1.96	5.64	7.19
contig03085:1:685:1 similar to Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type (Q7PPA5) Evalue: 9e-98	562	17 (13)	55.3	228	1.05	10.58	8.95
contig02754:347:1399:2 similar to Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type (Q7PPA5) Evalue: 1e-150	739	20 (11)	38.6	350	1.46	7.44	7.71
contig11608:1:909:2 similar to Plasma membrane calcium-transporting ATPase 4 (P23634) Evalue: 1e-102	148	3	10.9	302	0.62	0.84	0.63
contig06386:752:1527:2 similar to Plasma membrane calcium-transporting ATPase 3 (Q16720) Evalue: 1e-67	298	4	26.4	258	0.24	1.44	1.03
<b>iron transport</b>							
contig08407:69:1061:3 similar to Sideroflexin-3 (Q9JHY2) Evalue: 3e-106	290	10 (6)	23	330	0.42	0.84	1.18
contig02827:1:1595:3 similar to Melanotransferrin (P08582) Evalue: 5e-36	584	13 (12)	31.7	530		1.2	1.25
contig26835:204:534:3 similar to Ferritin, liver middle subunit (P85836) Evalue: 3e-17	230	6 (5)	38.2	110	6.46	2.25	1.57
contig25338:117:710:3 similar to Soma ferritin (P42577) Evalue: 9e-18	240	8 (5)	32.5	197	2.46	1.56	1.49
contig11630:1846:2601:1 similar to Soma ferritin (P42577) Evalue: 5e-71	406	8 (6)	29.1	251	1.92		0.56
contig06849:1:534:1 similar to Soma ferritin (P42577) Evalue: 2e-55	561	20 (9)	58.2	177	11.7		

<b>Protein transport</b>							
<b>COPE family.</b>							
contig27897:1:262:2 similar to Coatomer subunit epsilon (Q28104) Evalue: 8e-16	253	4	52.3	86	0.82	1.8	1.45
<b>COPG family.</b>							
contig26726:1:674:3 similar to Coatomer subunit gamma-2 (Q66JI9) Evalue: 7e-38	218	5 (4)	24.2	223	1.12	0.87	0.46
contig21179:1:442:3 similar to Coatomer subunit zeta-1 (P61924) Evalue: 1e-49	249	5 (3)	27.2	147	0.74		
contig20906:1:416:1 similar to Coatomer subunit gamma (P53620) Evalue: 4e-24	93	2	20.3	138	0.79	0.68	
contig20586:1:478:1 similar to Coatomer subunit beta~ (O55029) Evalue: 4e-39	40	1	5	159	0.41	0.38	
contig18962:1:2389:2 similar to Coatomer subunit alpha (P53621) Evalue: 0.0	673	12	20.1	795	1.47	1.72	0.91
contig18153:1:1867:2 similar to Coatomer subunit beta~ (Q5R664) Evalue: 0.0	364	9	17.2	622	0.75	1.24	0.63
contig17101:1:466:1 similar to Coatomer subunit alpha (Q8CIE6) Evalue: 2e-58	61	2	14.2	155	0.8	0.61	0.38
contig08624:1:837:3 similar to Coatomer subunit gamma (Q9QZE5) Evalue: 2e-91	188	3	18	278	0.73	0.47	0.98
<b>adaptor complexes medium subunit family. Delta-COP subfamily.</b>							
contig06493:1:1427:3 similar to Coatomer subunit delta (Q5XJY5) Evalue: 4e-144	332	7	23.2	475	1.11	1.11	0.78
contig05225:1:509:3 similar to Coatomer subunit epsilon (Q60445) Evalue: 3e-36	112	4 (3)	25.4	169			0.63
contig03994:94:3063:1 similar to Coatomer subunit beta (Q5R922) Evalue: 0.0	1239	23 (21)	29.2	989	0.41	1.19	0.49
<b>exportin family</b>							
contig00357:1:1325:3 similar to Exportin-1 (Q80U96) Evalue: 3e-154	149	3	7.5	440	0.21	0.21	
contig20690:1:2028:1 similar to Exportin-2 (Q9PTU3) Evalue: 1e-135	178	5	9.5	675	0.74	0.27	0.18
contig14970:1:594:1 similar to Exportin-2 (Q7SZC2) Evalue: 4e-27	189	3	22.3	197	0.62	0.51	
contig22968:1:1340:2 similar to Exportin-7 (Q5ZLT0) Evalue: 4e-143	98	2	4.9	446	0.25	0.19	0.12
contig27760:1:458:1 similar to Protein transport protein Sec61 subunit alpha-like 1 (Q90ZM2) Evalue: 4e-64	114	3	34.2	152	1.69	4.16	3.64
contig06044:1:1060:2 similar to Protein transport protein Sec61 subunit alpha-like 1 (Q90ZM2) Evalue: 1e-179	166	4	13.6	352	1.37	2.64	2.3
contig23239:932:3391:2 similar to Protein transport protein sec23 (Q5BGR9) Evalue: 0.0	736	14 (12)	19.5	819	0.45	0.79	0.38
contig19453:1:881:3 similar to Protein transport protein Sec24C (P53992) Evalue: 6e-59	92	3	12	292		0.33	
contig07781:1:1003:1 similar to Protein transport protein Sec24C (P53992) Evalue: 4e-109	156	3	12.9	334		0.28	
<b>NUP54 family</b>							
contig23247:1:1409:3 similar to Nucleoporin p54 (Q8BTS4) Evalue: 9e-46	99	2	6.2	468			0.13
contig12446:1:1813:2 similar to Nucleoporin NUP85 (Q4QQS8) Evalue: 3e-23	116	2	3.6	603		0.09	
contig03033:1:1390:2 similar to Nucleoporin GLE1 (Q4KLN4) Evalue: 3e-23	30	1	1.5	462			0.12
contig01482:1:1207:2 similar to Nucleoporin SEH1 (Q8R2U0) Evalue: 3e-108	83	2	9.2	401	0.15		
<b>synaptophysin/synaptobrevin family</b>							
contig24037:1:610:3 similar to Synaptoporin (Q8BGN8) Evalue: 1e-27	98	3 (2)	12.4	202	0.92	0.76	1.07
<b>Transmembrane proteins</b>							
contig04250:1:941:3 similar to Thioredoxin-related transmembrane protein 1 (Q0Z7W6) Evalue: 4e-44	147	4 (3)	12.5	312	1.02	0.86	0.76
contig16561:1:401:1 similar to Leucine-rich repeat transmembrane neuronal protein 3 (Q8BZ81) Evalue: 2e-10	79	2	10.5	133		0.49	0.49
contig25726:1:590:3 similar to Transmembrane protein 111 (Q7SXW4) Evalue: 1e-62	105	3	13.3	196		0.52	
contig24567:2523:3216:2 similar to Transmembrane protein 33 homolog (Q9XWV0) Evalue: 3e-30	163	4	17	230	0.4	0.74	
contig23936:1:1227:1 similar to Transmembrane protein C2orf18 (Q8N357) Evalue: 2e-97	151	3	9.1	408	0.66	0.52	0.3
contig22739:1:578:3 similar to Transmembrane protein 120A (Q8C1E7) Evalue: 3e-51	87	1	6.8	192		0.3	
contig20983:1:280:1 similar to Transmembrane protein 50A (Q9CXL1) Evalue: 7e-30	50	1	12.9	93	0.66		

contig19938:1:470:2 similar to Transmembrane protein 86A (Q3MHQ7) Evalue: 9e-09	44	1	5.8	156		0.57		
contig18428:1:607:2 similar to Transmembrane protein 97 (Q8VD00) Evalue: 5e-10	130	4 (3)	13.9	201	1.57	1.55	1.98	
contig18285:1:536:3 similar to Transmembrane protein 86B (Q3T0W0) Evalue: 6e-08	135	2	16.4	177		0.54	0.34	
contig03615:86:301:2 similar to UPF0197 transmembrane protein C11orf10 homolog (Q32P84) Evalue: 2e-10	51	1	12.5	72	0.86	1.29	1.29	
contig02190:1:482:3 similar to Transmembrane protein 194A (O14524) Evalue: 1e-13	174	3	27	159	0.64			
contig02001:99:2246:3 similar to Transmembrane protein 214 (A4FV45) Evalue: 1e-24	586	13 (12)	24.3	715	0.94	0.77	0.42	
contig01477:1:451:2 similar to Transmembrane protein 69 (Q3KQJ0) Evalue: 2e-10	44	1	4.7	149	0.2	2.2	2.05	
<b>nonaspanin (TM9SF) family.</b>								
contig10176:168:613:3 similar to Transmembrane 9 superfamily member 2 (P58021) Evalue: 2e-38	66	2	12.2	148		0.63		
contig25673:1:1525:2 similar to Transmembrane 9 superfamily member 2 (Q5R8Y6) Evalue: 0.0	76	2	4.5	507	0.24	0.48	0.18	
contig23087:273:2027:3 similar to Transmembrane 9 superfamily member 3 (Q9ET30) Evalue: 0.0	35	1	1.9	584	0.1	0.15	0.1	
contig09206:225:1888:3 similar to Transmembrane 9 superfamily member 4 (Q8BH24) Evalue: 2e-179	34	1	1.6	554		0.2	0.16	
<b>EMP24/GP25L family.</b>								
contig04478:168:863:3 similar to Transmembrane emp24 domain-containing protein 1 (Q28BQ6) Evalue: 3e-28	116	3	11.7	231	0.4	0.86	0.27	
contig18001:1:471:1 similar to Transmembrane emp24 domain-containing protein 2 (P49020) Evalue: 1e-52	269	6	39.7	156	1.43	2.43	1.01	
contig23762:106:762:1 similar to Transmembrane emp24 domain-containing protein 7 (Q9Y3B3) Evalue: 2e-53	209	6 (3)	17.8	219	0.76	0.89	0.97	
contig17283:1:716:2 similar to Transmembrane emp24 domain-containing protein 9 (Q9BVK6) Evalue: 2e-48	194	5 (4)	17.6	238	0.41	0.71	0.65	
contig02400:1:762:1 similar to Transmembrane emp24 domain-containing protein 10 (Q5E971) Evalue: 3e-62	350	13 (7)	25.7	253	2.55	2.15	1.96	
<b>Channels</b>								
contig17293:1:839:3 similar to Cullin-5 (Q29425) Evalue: 3e-59	90	2	11.2	278	0.34	0.32		
contig04295:229:1159:1 similar to Inositol 1,4,5-trisphosphate receptor type 1 (Q14643) Evalue: 1e-84	39	1	4.2	310	0.18	0.09	0	
<b>MIP/aquaporin (TC 1.A.8) family.</b>								
contig26144:1190:2191:2 similar to Aquaporin-9 (O43315) Evalue: 8e-43	48	1	2.7	333		0.18		
contig24282:1:693:1 similar to Aquaporin-10 (Q96PS8) Evalue: 3e-39	99	1	7	230		0.26		
contig17421:247:1275:1 similar to Aquaporin-4 (Q5I4F9) Evalue: 2e-36	271	11 (5)	15.2	342	15.82	5.52	6.56	
contig04424:252:1300:3 similar to Aquaporin-10 (Q96PS8) Evalue: 4e-56	430	7 (6)	26.9	349	1.8	4.67	4.1	
contig01013:1:991:2 similar to Aquaporin-3 (Q08DE6) Evalue: 1e-39	39	1	8.2	329			0.18	
contig26932:43:801:1 similar to Chloride intracellular channel exc-4 (Q8WQA4) Evalue: 2e-50	304	6	34.5	252	1.08	1.11	0.81	
contig26155:1:131:3 similar to Voltage-dependent anion-selective channel protein 1 (Q60932) Evalue: 4e-09	115	8 (1)	42.9	42	7.25	20.41	12.9	
contig23401:118:845:1 similar to Voltage-dependent anion-selective channel protein 2 (P81004) Evalue: 2e-41	892	43 (16)	76.9	242	10.78	22.08	18.67	
contig22692:232:1080:1 similar to Trimeric intracellular cation channel type B (Q7ZVP8) Evalue: 4e-39	133	4 (3)	14.5	282		0.45	0.32	
<b>Ligand-gated ionic channel family</b>								
contig25798:1:638:3 similar to Neuronal acetylcholine receptor subunit alpha-4 (Q5IS77) Evalue: 6e-11	174	3	21.8	211	0.49			
contig25495:79:789:1 similar to Acetylcholine receptor subunit alpha-like 2 (P17644) Evalue: 7e-09	100	3	11.9	236		0.42	0.54	
contig20272:1:245:1 similar to Neuronal acetylcholine receptor subunit beta-2 (P09484) Evalue: 3e-05	103	5 (2)	24.7	81	1.28	3.08	7.14	
contig19129:1:846:1 similar to Neuronal acetylcholine receptor subunit beta-3 (Q5IS75) Evalue: 8e-10	58	2	6.7	282		0.32		
contig17705:184:1038:1 similar to Acetylcholine receptor subunit alpha-L1 (P23414) Evalue: 4e-23	168	5 (3)	11.6	284	1.25	0.89	0.98	
contig10452:143:865:2 similar to Neuronal acetylcholine receptor subunit alpha-6 (Q9R0W9) Evalue: 7e-10	82	2	10.4	240	0.26			
contig09320:131:802:2 similar to Acetylcholine receptor subunit gamma (P02714) Evalue: 9e-12	146	5 (4)	17	223	0.26	1.72	2.1	
contig02467:116:940:2 similar to Neuronal acetylcholine receptor subunit eat-2 (Q9U298) Evalue: 4e-08	141	4	15.3	274	0.62	0.87	0.51	
contig01978:53:763:2 similar to Acetylcholine receptor subunit gamma (P05376) Evalue: 3e-14	43	1	9.7	236		0.24	0.38	



**AAA ATPase family.**

contig10458:1:210:3 similar to Transitional endoplasmic reticulum ATPase (P46462) Evalue: 4e-27	134	2	44.9	69	3.96	5.94	4.88
contig10723:1:109:1 similar to Transitional endoplasmic reticulum ATPase (Q6GL04) Evalue: 3e-15	38	1	38.9	36		1.88	
contig20935:1:608:1 similar to Transitional endoplasmic reticulum ATPase (P23787) Evalue: 7e-81	207	5	28.2	202	0.87	2.38	1.92
contig21090:1:317:3 similar to Transitional endoplasmic reticulum ATPase (P23787) Evalue: 2e-27	206	4	46.2	104	2.56	2.05	1.76
contig13332:1:832:3 similar to Transitional endoplasmic reticulum ATPase (P23787) Evalue: 6e-99	92	2	11.2	276		0.22	0.35

**others**

contig26145:1:1544:2 similar to Sodium-independent sulfate anion transporter (Q86WA9) Evalue: 3e-74	57	1	2.5	514	0.12		
contig26126:94:1870:1 similar to Monocarboxylate transporter 12 (Q6ZSM3) Evalue: 1e-30	213	5	11.3	592	0.43	0.54	0.7
contig26110:1:1187:3 similar to Magnesium transporter protein 1 (Q9CQY5) Evalue: 2e-88	123	3	8.9	394		0.31	0.22
contig24817:1:584:3 similar to Ammonium transporter 2 (Q9BLG3) Evalue: 2e-05	69	1	11.3	194	0.32		
contig24486:255:2128:3 similar to Sodium- and chloride-dependent glycine transporter 2 (A7Y2X0) Evalue: 3e-96	76	2	3.8	624		0.1	
contig22182:1:826:2 similar to Zinc transporter SLC39A7 (Q92504) Evalue: 1e-08	43	1	6.2	275	0.22	0.22	
contig21487:545:1370:2 similar to Excitatory amino acid transporter 3 (P51906) Evalue: 2e-21	100	2	9.5	275		0.8	0.68
contig14683:115:457:1 similar to Sodium bicarbonate cotransporter 3 (Q8BTY2) Evalue: 5e-18	108	2	16.7	114		1.12	0.52
contig10433:1:1019:3 similar to Excitatory amino acid transporter 1 (P24942) Evalue: 5e-82	102	2	14.8	338		0.37	0.18
contig09658:1:461:2 similar to Ammonium transporter 1 member 2 (Q6K9G1) Evalue: 2e-24	63	2 (1)	7.2	153	0.84		
contig09050:1:1724:3 similar to Canalicular multispecific organic anion transporter 1 (Q92887) Evalue: 0.0	105	2	6.1	573		0.2	0.1
contig07527:162:1012:3 similar to Mitochondrial folate transporter/carrier (Q8BMG8) Evalue: 1e-53	142	3	11.3	283	0.22	0.35	0.5
contig04135:1:1511:3 similar to ABC transporter F family member 1 (Q9FJH6) Evalue: 0.0	106	3	7	502		0.18	0.24
contig02938:228:1706:3 similar to Solute carrier family 2, facilitated glucose transporter member 1 (P27674) Evalue: 5e-108	63	2	4.1	492	0.42	0.54	0.42
contig01729:1:437:3 similar to Sodium- and chloride-dependent creatine transporter 1 (P48029) Evalue: 1e-28	51	1	6.2	144		0.4	
contig00457:317:1870:2 similar to Ammonium transporter Rh type B (Q7T070) Evalue: 9e-101	152	4 (3)	5.8	517		0.84	1.47

**Structural constituent of cytoskeleton****spectrin family.**

contig21858:1:970:2 similar to Spectrin beta chain (Q00963) Evalue: 4e-68	580	13 (12)	37.2	323	0.45	7.96	4.64
contig26171:1:360:1 similar to Spectrin beta chain (Q00963) Evalue: 2e-13	218	4	41.2	119		6.12	4.56
contig17126:1:561:2 similar to Spectrin beta chain (Q00963) Evalue: 9e-80	359	7 (5)	34.9	186		7.21	4.24
contig13568:1:351:2 similar to Spectrin beta chain (Q00963) Evalue: 6e-28	231	3	31	116		3.21	3.36
contig11249:1:845:3 similar to Spectrin beta chain (Q00963) Evalue: 2e-37	257	5 (4)	22.4	281		2.27	2.17
contig01265:220:662:1 similar to Spectrin beta chain (Q00963) Evalue: 2e-45	149	4	29.9	147	1	4.84	4.42
contig13751:1:318:1 similar to Spectrin alpha chain, brain (P16086) Evalue: 8e-23	106	3	30.2	106		4.02	2.03
contig04108:1:735:1 similar to Spectrin alpha chain (P13395) Evalue: 2e-30	194	5	18	245		2.45	2.26
contig20608:1:663:2 similar to Spectrin alpha chain (P13395) Evalue: 2e-53	267	7	28.6	220		4.63	3.14
contig03159:331:893:1 similar to Spectrin alpha chain (P13395) Evalue: 1e-69	212	6 (4)	27.3	187		2.84	1.96
contig25376:1:1382:3 similar to Spectrin alpha chain, brain (P16546) Evalue: 0.0	611	11	33.3	459	0.19	4.52	4.29
contig03159:1:224:3 similar to Spectrin alpha chain, brain (P16546) Evalue: 3e-10	52	1	13.7	73	1.17	3.12	

**tropomodulin family.**

contig18006:1:304:2 similar to Tropomodulin (O01479) Evalue: 1e-18	223	3	38	100		1.2	1.87
contig23584:13:773:1 similar to Tropomodulin (O01479) Evalue: 4e-48	71	2	10.3	253		0.25	

<b>septin family</b>								
contig02708:629:2158:2 similar to Septin-2 (P54359) Evalue: 6e-100	95	2	5.9	509	0.18			
contig25517:226:1350:1 similar to Septin-2A (Q5BKN4) Evalue: 1e-109	294	8 (5)	18.7	374	0.55			
contig26638:1:767:3 similar to Probable actin-related protein 2/3 complex subunit 2 (Q9VIM5) Evalue: 1e-105	172	4	15.4	254	0.89	0.53	0.89	
<b>actin family</b>								
contig26506:1:175:3 similar to Actin-11 (P41341) Evalue: 6e-27	97	12 (2)	47.4	57	9.85	9.55	5.76	
contig06736:134:244:2 Actin-related protein 2/3 complex subunit 3 [Caligus rogercresseyi] (GI_225708752) Evalue: 2e-07	86	2	62.2	37	1.88	2.76		
contig25399:201:803:3 similar to Actin-10 (Q54GX7) Evalue: 2e-108	674	27 (13)	62	200		6.49	2.73	
contig25399:1:211:2 similar to Actin, clone 302 (P18602) Evalue: 2e-29	60	1	42	69		0.9		
contig23341:69:1196:3 similar to Actin, alpha skeletal muscle (P68139) Evalue: 0.0	740	66 (15)	46.9	375	1.46	4.77	3.49	
contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	461	87 (7)	74.6	118	25.87	75.63	89.85	
contig17982:1:724:2 similar to Actin-5C (P10987) Evalue: 1e-138	1143	182 (16)	76.2	240	36.13	192.6	101.14	
contig11420:186:337:3 similar to Actin (Q9UVX4) Evalue: 1e-25	99	10 (2)	54	50	5.26	19.32	9.8	
contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	1153	183 (16)	57.4	319	18.86	62.7	52.95	
contig24196:132:1388:3 similar to Actin-related protein 3 (P32392) Evalue: 0.0	367	9 (7)	28.7	418	0.31	0.7	0.59	
contig01465:1:1216:2 similar to Actin-related protein 2-B (Q7ZXV3) Evalue: 2e-180	222	6 (4)	11.1	405	0.32	0.41	0.23	
contig22775:1:327:1 similar to Actin-related protein 2/3 complex subunit 3 (O15145) Evalue: 2e-38	33	1	6.5	108	0.54			
contig17253:1:906:1 similar to Actin-related protein 2/3 complex subunit 1A (Q9ROQ6) Evalue: 3e-86	54	2	7.6	302			0.21	
contig08163:251:575:2 similar to Actin-related protein 2/3 complex subunit 4 (P59998) Evalue: 8e-49	73	2	18.5	108		0.62	0.62	
contig16483:1:1380:1 similar to Actin-like protein 6A (O96019) Evalue: 6e-115	134	3	7.8	459	0.26	0.2		
<b>WD repeat AIP1 family</b>								
contig24474:152:1993:2 similar to Actin-interacting protein 1 (Q9VU68) Evalue: 0.0	796	19 (17)	38	613	0.87	1.37	1.22	
<b>alpha-actinin family</b>								
contig13124:1:1993:2 similar to Alpha-actinin-2 (Q3ZC55) Evalue: 0.0	1826	44 (35)	64.9	663	0.09	6.42	3.81	
contig03818:1:573:2 similar to Alpha-actinin, sarcomeric (P18091) Evalue: 9e-103	824	20 (12)	74.2	190		15.07	8.6	
<b>tropomyosin family</b>								
contig28247:1:128:3 similar to Tropomyosin (Q9GZ69) Evalue: 2e-05	135	4 (3)	38.1	42		4.21	4.94	
contig22479:1:399:3 similar to Tropomyosin (Q95VA8) Evalue: 1e-38	560	20 (11)	58.3	132	1.04	19.98	22.77	
contig21354:1:133:1 similar to Tropomyosin-2 (Q1HPQ0) Evalue: 3e-05	144	3	34.1	44		5.88	8.52	
contig06886:1:366:1 similar to Tropomyosin, muscle (P15846) Evalue: 1e-39	350	12 (7)	52.9	121	0.54	5.17	4.03	
<b>tubulin family</b>								
contig28259:1:544:2 similar to Tubulin alpha-1 chain (Q25008) Evalue: 8e-88	197	9 (4)	25.6	180	1.14		0.58	
contig25921:1:1436:3 similar to Tubulin beta-1 chain (O17449) Evalue: 0.0	1577	65 (21)	56.6	477	9.1	11.18	8.19	
contig24968:1:1457:3 similar to Tubulin beta-1 chain (O17449) Evalue: 0.0	1424	64 (20)	53.5	484	15.24	9.09	7.92	
contig24792:1:405:3 similar to Tubulin alpha-1C chain (P68365) Evalue: 2e-53	288	12 (5)	51.5	134	2.44			
contig20806:52:1386:1 similar to Tubulin beta-1 chain (O17449) Evalue: 0.0	431	16 (8)	18.9	444		1.02	0.78	
contig20065:1:1271:3 similar to Tubulin beta-1 chain (O17449) Evalue: 0.0	754	36 (13)	35.1	422	10.44	3.66	1.92	
contig13765:1:413:3 similar to Tubulin beta chain (P02556) Evalue: 2e-61	124	6 (2)	19.1	136		0.79		
contig06240:1:463:2 similar to Tubulin-folding cofactor B (Q9D1E6) Evalue: 4e-12	254	6 (5)	42.9	154	2.91	1.92	2.12	
contig05639:1:407:3 similar to Tubulin alpha-2 chain (P34690) Evalue: 2e-59	69	4 (2)	17.8	135		0.82		
contig04581:1:1472:3 similar to Tubulin alpha-4A chain (P68368) Evalue: 0.0	1174	55 (16)	42.9	489	11.74	8.33	5.62	

contig04046:37:1419:1 similar to Tubulin gamma-1 chain (P83887) Evalue: 0.0	90	2	5	460	0.13		
<b>Motor proteins</b>							
contig27193:192:1466:3 similar to Cytoplasmic dynein 1 light intermediate chain 2 (Q6PDL0) Evalue: 6e-83	277	6	20.3	424	0.39	0.49	0.39
contig24984:639:2159:3 similar to Cytoplasmic dynein 1 intermediate chain 2 (O88487) Evalue: 3e-129	156	3	8.3	506	0.25	0.18	
contig23639:1:1930:2 similar to Dynein heavy chain, cytoplasmic (P37276) Evalue: 2e-179	179	5	10.4	643	0.44		
contig16346:1:458:3 similar to Dynein heavy chain, cytoplasmic (P37276) Evalue: 1e-28	87	2	13.9	151	0.8		
contig21656:179:382:2 similar to Dynein light chain LC6, flagellar outer arm (O02414) Evalue: 1e-31	219	4 (3)	58.8	68	3.12		
<b>Muscle proteins</b>							
contig25810:71:1195:2 similar to Troponin T (Q9XZ71) Evalue: 4e-31	855	39 (20)	39.8	374	1.15	6.8	6.33
contig18586:44:1159:2 similar to Troponin T (Q9XZ71) Evalue: 1e-23	419	10 (9)	24.3	371	0.24	1.66	1.7
contig23852:3162:3608:1 similar to Troponin I (P36188) Evalue: 2e-23	595	35 (9)	43.2	148	4.97	32.94	22.06
contig22030:105:572:3 similar to Troponin C (P15159) Evalue: 1e-59	133	3	19.4	155	0.69		
contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	2744	111 (42)	62.4	556	2.07	62.28	47.74
contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	721	20 (9)	56.2	128	0.88	25.71	24.98
contig27485:90:265:3 similar to Myosin heavy chain, muscle (P05661) Evalue: 1e-08	111	3	36.2	58			5.22
contig26256:1:544:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-37	619	13 (7)	43.1	181	0.88	30.15	25.03
contig26128:1:2657:2 similar to Myosin-3 (P12844) Evalue: 0.0	2180	67 (36)	49.3	885	0.72	22.39	19.56
contig25968:1:335:3 similar to Myosin-6 (Q02566) Evalue: 8e-18	227	3 (2)	29.7	111		6.67	6.14
contig24776:1:504:1 similar to Myosin-2 essential light chain (P54357) Evalue: 1e-46	138	4	28.1	167	1.1		
contig22799:1:383:1 similar to Myosin-2 essential light chain (P54357) Evalue: 3e-44	28	1	7.1	127	0.46		
contig17935:1:1428:1 similar to Myosin heavy chain, embryonic smooth muscle isoform (Q99105) Evalue: 1e-76	38	1	1.9	475		0.12	
contig16627:471:1223:3 similar to Myosin, essential light chain (P53014) Evalue: 5e-31	150	4 (3)	16	250	1.4	0.51	0.73
contig15547:1:808:2 similar to Myosin heavy chain, non-muscle (Q99323) Evalue: 2e-132	37	1	3.7	269	0.33		
contig15494:1:257:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 8e-18	331	8 (4)	42.4	85	1.94	29.04	29.07
contig14962:1:769:2 similar to Myosin heavy chain, muscle (P05661) Evalue: 6e-58	1261	35 (21)	64.7	255	3.7	62.42	54.71
contig14012:1:1036:2 similar to Myosin heavy chain, non-muscle (Q99323) Evalue: 4e-103	393	8	25.2	345	0.24	1.3	0.71
contig13127:1:930:1 similar to Myosin-7 (Q91Z83) Evalue: 1e-116	1071	33 (16)	64.5	310	3.24	46.57	34.4
contig13126:1:120:2 similar to Myosin-11 (Q63862) Evalue: 1e-04	103	1	38.5	39		4.85	4.85
contig13125:303:769:3 similar to Myosin heavy chain, muscle (P05661) Evalue: 2e-52	295	7 (6)	52.3	155	0.38	13.56	16.63
contig10445:1:469:2 similar to Myosin heavy chain, muscle (P05661) Evalue: 8e-40	295	8 (6)	26.9	156		8.82	6.07
contig08542:152:670:2 similar to Probable myosin regulatory light chain (Q09510) Evalue: 4e-50	730	26 (11)	76.2	172	3.43	11.87	10.27
contig05208:1:597:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 3e-50	810	21 (12)	62.3	199	1.07	48.82	41.35
contig02107:1:417:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-35	526	16 (9)	57.6	139	0.42	45.53	49.63
contig01345:1:144:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 2e-08	233	4	54.2	48		43.96	51.66
contig00641:1:549:1 similar to Myosin heavy chain, striated muscle (P24733) Evalue: 3e-50	553	15 (10)	53	183		34.33	35.67
contig00571:1:108:2 similar to Myosin-3 (P12844) Evalue: 4e-08	144	5 (4)	71.4	35		31.51	66.84
<b>Pathogenesis-related protein</b>							
<b>thaumatin family.</b>							
contig03028:130:858:1 similar to Pathogenesis-related protein 5 (P28493) Evalue: 1e-19	128	2	12	242		0.26	1
contig05319:82:714:1 similar to Pathogenesis-related protein 5 (P28493) Evalue: 7e-35	190	4	24.8	210		0.7	0.49
contig24159:1:208:2 similar to Pathogenesis-related protein 5 (P28493) Evalue: 9e-15	137	10 (3)	45.6	68		4.48	3.39

contig25505:1:208:2 similar to Pathogenesis-related protein 5 (P28493) Evalue: 6e-15	136	10 (3)	45.6	68	3.39	4.48
contig02384:1:343:2 similar to Pathogenesis-related protein 5 (P28493) Evalue: 4e-19	110	5 (3)	23.9	113		1.04
contig28090:82:614:1 similar to Pathogenesis-related protein 5 (P28493) Evalue: 7e-25	221	13 (3)	20.9	177	0.79	2.29
<b>Chitinases</b>						
contig28310:68:610:2 similar to Chitinase D (P27050) Evalue: 1e-08	233	6 (4)	41.1	180	1.15	1.51
contig26897:19:1285:1 similar to Endochitinase (P36362) Evalue: 1e-41	437	7	27.5	422	0.75	0.58
contig22564:1:404:3 similar to Acidic endochitinase SP2 (P42820) Evalue: 3e-16	463	16 (8)	57.9	133	2.13	7.35
contig20760:1:720:3 similar to Chitinase 5 (Q7Y1Z0) Evalue: 2e-19	282	6 (5)	25.5	239	1.6	0.84
contig28279:1:702:1 similar to Plasma alpha-L-fucosidase (Q6AYS4) Evalue: 2e-52	371	10 (7)	29.2	233	2.06	2.21
contig05782:1:148:1 similar to Plasma alpha-L-fucosidase (Q5RFI5) Evalue: 4e-09	38	2 (1)	22.4	49	2.38	1.95
contig05498:1:1566:3 similar to Plasma alpha-L-fucosidase (Q6AYS4) Evalue: 2e-125	83	2	5.6	521	0.22	0.15
contig18379:1:612:3  AGAP009479-PA [Anopheles gambiae str (XP_001230737.2)] Evalue: 2e-22	523	26 (8)	48.8	203	1	6.58
contig04768:1:376:2  conserved hypothetical protein [Culex quinquefasciatus] (XP_001865643.1) Evalue: 1e-18	164	3 (2)	28.2	124	1.08	1.08
contig01202:555:721:3  PREDICTED: similar to ENSANGP0000013458 [Nasonia vitripennis] (XP_001599617.1) Evalue: 7e-06	159	7 (2)	41.8	55	7.02	12.14
contig06721:1:451:2  AGAP009479-PA [Anopheles gambiae str (XP_001230737.2)] Evalue: 1e-17	133	7 (2)	16.8	149	3.64	4.8
contig12042:1:796:2  AGAP008123-PA [Anopheles gambiae str (XP_317336.3)] Evalue: 7e-74	267	6	33.3	264	0.83	
contig18676:129:680:3  PREDICTED: similar to CG14301-PA isoform 1 [Apis mellifera] (XP_392551.2) Evalue: 1e-17	143	3	27.9	183	0.58	1.3
contig01761:290:940:2  hypothetical protein Phum_PHUM355660 [Pediculus humanus corporis] (XP_002427954.1) Evalue: 9e-18	38	2 (1)	5.1	217	0.56	0.52
contig06213:292:1380:1  AGAP009479-PA [Anopheles gambiae str (XP_001230737.2)] Evalue: 9e-20	90	2	8	362	0.34	0.43
contig13408:1:955:1  conserved hypothetical protein [Culex quinquefasciatus] (XP_001865643.1) Evalue: 1e-12	53	3 (1)	3.5	318		0.18
contig27723:142:903:1  PREDICTED: similar to CG14608-PA [Apis mellifera] (XP_395554.2) Evalue: 3e-20	58	2	9.9	253	0.25	0.99
contig07253:7:561:1  GH19216 [Drosophila grimshawi] (XP_001990223.1) Evalue: 1e-17	173	4 (3)	25	184	1.3	1.24
contig00352:1:376:1  AGAP009479-PA [Anopheles gambiae str (XP_001230737.2)] Evalue: 3e-20	217	3	40	125	1.69	0.48
contig11457:1:1039:2  PREDICTED: similar to CG14608 CG14608-PB, partial [Acyrtosiphon pisum] (XP_001942936.1) Evalue: 3e-15	133	4 (2)	6.6	346	0.72	
contig13408:1:955:1  conserved hypothetical protein [Culex quinquefasciatus] (XP_001865643.1) Evalue: 1e-12	52	1	3.5	318	0.27	
contig07690:429:1916:3  hypothetical protein Bm1_29410 [Brugia malayi] (XP_001897324.1) Evalue: 4e-15	131	2	5.7	495	0.18	0.12
contig17011:111:854:3  chitinase A [Equisetum arvense] (GI_257074554) Evalue: 1e-05	127	3 (2)	15.8	247	0.56	0.73
contig07753:47:529:2  chitinase A [Pteris ryukyuensis] (GI_110556116) Evalue: 3e-05	190	3	15	160	0.43	1.34
contig12046:1:481:2  GK18229 [Drosophila willistonii] (XP_002066317.1) Evalue: 4e-35	189	5	46.5	159	1.25	
contig03173:1:473:1  hypothetical protein sce4008 [Sorangium cellulosum 'So ce 56'] (YP_001614648.1) Evalue: 7e-58	189	4	28.7	157	1.02	0.7
<b>LEA protein</b>						
contig00574:89:529:2  Late embryogenesis abundant protein [Alteromonas macleodii ATCC 27126] (ZP_04714463.1) Evalue: 4e-05	733	23 (14)	61.9	147	10.19	15.19
<b>Translational proteins</b>						
contig27961:1:347:1 similar to Translation initiation factor eIF-2B subunit alpha (Q99LC8) Evalue: 2e-14	62	2	20	115	0.86	1.02
contig27468:1:1259:3 similar to Eukaryotic translation initiation factor 3 subunit D (Q0ZB77) Evalue: 7e-117	206	3	9.8	418	0.67	0.43
contig27051:11:2035:2 similar to Eukaryotic translation initiation factor 5 (Q9VXK6) Evalue: 1e-99	196	4	7.6	674	0.18	0.09

## Supplementary

contig26861:1:345:3 similar to Eukaryotic translation initiation factor 4 gamma 1 (Q04637) Evalue: 2e-14	130	2	29.8	114	0.54	0.61	0.61
contig26081:1:614:2 similar to Translation initiation factor eIF-2B subunit alpha (Q14232) Evalue: 3e-59	160	5 (3)	19.1	204		0.74	
contig25843:96:1024:3 similar to Eukaryotic translation initiation factor 2 subunit 1 (Q5R493) Evalue: 4e-88	574	12 (10)	35.9	309	2.76	3.42	3.44
contig25734:1:558:1 similar to Eukaryotic translation initiation factor 3 subunit I (Q16K15) Evalue: 3e-51	280	4	30.3	185	1.48	0.83	1.64
contig25600:215:1093:2 similar to Eukaryotic translation initiation factor 3 subunit F (O00303) Evalue: 7e-60	410	11 (8)	31.2	292	1.95	2.32	1.24
contig25431:91:567:1 similar to Eukaryotic translation initiation factor 1A, Y-chromosomal (O14602) Evalue: 7e-44	103	4 (2)	13.3	158	0.41		
contig25423:124:1422:1 similar to Translation initiation factor eIF-2B subunit delta (P41111) Evalue: 2e-102	121	3	8.1	432	0.14	0.22	0.31
contig25369:1:456:1 similar to Eukaryotic translation initiation factor 3 subunit E (A5AAA4) Evalue: 1e-12	189	4	28.3	152	1.43	1.01	1.01
contig24591:1:820:2 similar to Eukaryotic translation initiation factor 2 subunit 2 (Q99L45) Evalue: 5e-63	386	7	23.8	273	1.7	1.25	1.36
contig24241:1:259:2 similar to Eukaryotic translation initiation factor 3 subunit B (Q8JZQ9) Evalue: 3e-12	183	2	31.8	85		0.78	0.78
contig23557:134:1258:2 similar to Translation initiation factor eIF-2B subunit beta (Q90511) Evalue: 6e-96	104	3	8.6	374	0.36	0.26	0.16
contig23539:279:2824:3 similar to Eukaryotic translation initiation factor 5B (Q05D44) Evalue: 0.0	158	4 (3)	4.4	848		0.18	0.07
contig23193:132:1322:3 similar to Eukaryotic translation initiation factor 3 subunit M (Q6DK91) Evalue: 5e-53	647	13 (10)	36.1	396	1.4	1.86	1.3
contig22178:97:470:1 similar to Eukaryotic translation initiation factor 4E (P48598) Evalue: 2e-19	228	5 (4)	44.4	124	1.39	1.54	1.54
contig22102:137:561:2 similar to Eukaryotic translation initiation factor 3 subunit I (Q66J51) Evalue: 1e-38	173	3	26.2	141	0.78	0.78	0.78
contig20642:1:1099:2 similar to Eukaryotic translation initiation factor 3 subunit A (Q173M7) Evalue: 1e-12	77	2	11.7	366	0.24	0.56	0.48
contig19871:1:1549:2 similar to Eukaryotic translation initiation factor 2 subunit 3 (P81795) Evalue: 0.0	450	9	23.9	515	0.9	1.19	0.78
contig19339:1:692:3 similar to Translation initiation factor eIF-2B subunit epsilon (P47823) Evalue: 4e-18	80	2	10.4	230			0.27
contig18822:1:297:1 similar to Eukaryotic translation initiation factor 3 subunit E (A7RWP6) Evalue: 1e-13	332	7 (4)	73.7	99	1.92	2.77	3.92
contig18763:98:844:2 similar to Eukaryotic translation initiation factor 3 subunit C (B4HMY3) Evalue: 1e-33	172	4	16.9	249		0.55	0.24
contig18604:1:223:2 similar to Eukaryotic translation initiation factor 3 subunit L (Q7T2A5) Evalue: 1e-17	123	4 (3)	34.2	73	0.8	1.74	
contig18404:1:444:2 similar to Eukaryotic translation initiation factor 3 subunit H (Q9GV27) Evalue: 7e-16	51	1	11.6	147	1.49	0.4	0.44
contig18403:1:742:2 similar to Eukaryotic translation initiation factor 3 subunit H (A7SA47) Evalue: 4e-56	353	7	40.2	246	2.19	1.71	1.69
contig18211:1:768:1 similar to Eukaryotic translation initiation factor 3 subunit J (Q6INR1) Evalue: 1e-17	65	2	6.7	255	0.24	0.24	
contig12885:1:1387:2 similar to Eukaryotic translation initiation factor 3 subunit C (Q6P1V4) Evalue: 4e-127	415	8	25.3	462	0.49	0.92	0.55
contig12423:56:375:2 similar to Eukaryotic translation initiation factor 4B (P23588) Evalue: 1e-05	93	2	32.1	106	0.28		
contig12004:57:410:3 similar to Protein translation factor SUI1 homolog (P42678) Evalue: 1e-40	187	4 (3)	19.7	117	2.06		
contig08308:1:904:1 similar to Translation initiation factor eIF-2B subunit epsilon (Q8CHW4) Evalue: 1e-61	76	2	8.6	301			0.2
contig07751:1:531:2 similar to Eukaryotic translation initiation factor 3 subunit D (Q6TH15) Evalue: 7e-22	33	1	5.1	176			0.34
contig07646:1:1257:3 similar to Eukaryotic translation initiation factor 3 subunit A (A2VD00) Evalue: 7e-111	155	5 (3)	7.4	418	0.65	0.71	0.62
contig06785:1:330:1 similar to Probable eukaryotic translation initiation factor 3 subunit G (A8WLV5) Evalue: 4e-16	123	2	25.7	109	1.09	1.54	0.56
contig05141:83:409:2 similar to Eukaryotic translation initiation factor 3 subunit L (Q8AVJ0) Evalue: 2e-20	85	2	17.4	109		0.61	
contig03261:1:705:1 similar to Eukaryotic translation initiation factor 3 subunit E-B (Q1LUA8) Evalue: 4e-78	248	8 (5)	26	235	1.17	1.09	0.88
contig02684:38:859:2 similar to Translation initiation factor eIF-2B subunit gamma (P70541) Evalue: 3e-50	96	2	9.9	273			0.22
contig02658:79:561:1 similar to Eukaryotic translation initiation factor 5A (P62925) Evalue: 2e-63	192	8 (5)	40	160	9.48	2.55	1.38
contig02513:1:648:1 similar to Eukaryotic translation initiation factor 3 subunit K (Q28C65) Evalue: 1e-21	313	11 (6)	37.5	216	1.96	2.34	1.1
contig02032:933:1673:2 similar to Eukaryotic translation initiation factor 6 (P56537) Evalue: 2e-115	495	15 (9)	57.3	246	2.15	3.3	1.81
contig01985:1:275:1 similar to Eukaryotic translation initiation factor 3 subunit L (Q8AVJ0) Evalue: 7e-12	212	6 (3)	49.5	91	3.52	3.52	5.24
contig01429:234:1822:3 similar to Eukaryotic translation initiation factor 3 subunit B (Q8JZQ9) Evalue: 6e-122	415	11 (8)	17.6	529	0.37	0.77	0.8
contig01246:150:1179:3 similar to Eukaryotic translation initiation factor 2A (Q4QRJ7) Evalue: 5e-50	102	2	9	343			0.18
contig00173:1:607:2 similar to Eukaryotic translation initiation factor 4 gamma 1 (Q04637) Evalue: 1e-23	149	4	24.3	202		0.69	0.48

contig00873:1:333:1 similar to Translationally-controlled tumor protein homolog (Q60FS1) Evalue: 1e-32	335	15 (6)	70	110	3.22	6.34	4.96
contig21335:1:161:1 similar to Translationally-controlled tumor protein homolog (Q5MIP6) Evalue: 3e-15	88	3 (1)	22.6	53	1.22	2.44	1.83
<b>Histones</b>							
contig24583:1:349:2 similar to Histone H2A.x (P16104) Evalue: 8e-24	322	12 (5)	60.9	115	20.44	4.48	4.19
contig20910:105:416:3 similar to Histone H4 (P62799) Evalue: 9e-40	87	2	17.5	103	37.24	3.27	0.99
contig19209:1:376:3 similar to Histone H2A.V (P08991) Evalue: 5e-54	99	2	12.9	124	4.19	1.2	1.2
contig18253:102:232:3 similar to Histone H2A (P35061) Evalue: 3e-05	43	1	20.9	43	3.08	1.54	
contig17326:97:498:1 similar to Histone H2B (P17271) Evalue: 2e-44	96	3 (2)	18	133	12.67	0.53	
contig07813:1:482:3 similar to Histone H3.2 (Q5MYA4) Evalue: 3e-52	55	2	9.4	159	0.82		
contig06818:1:834:1 similar to Histone H3.3 (P84247) Evalue: 2e-73	209	6 (4)	19.5	277	1.77		
contig06014:56:460:2 similar to Histone H2A.1 (A5DBG4) Evalue: 4e-44	58	3 (2)	11.9	134	1.86	0.53	
contig05159:104:490:2 similar to Histone H2B 7 (POC1H5) Evalue: 2e-47	203	10 (5)	32	128	4.86		
contig02517:56:302:2 similar to Histone H2B.1 (Q6BRG2) Evalue: 3e-16	144	12 (2)	30.5	82	8.11		1.14
contig02313:281:1393:2 similar to Histone H1-delta (P15870) Evalue: 2e-09	120	3 (2)	6.8	370	0.34		
contig00384:1:500:3 similar to Histone H2A (Q5KMT5) Evalue: 5e-26	115	2	12.1	165	1.12	0.39	0.39
<b>Proteases</b>							
<b>AAA ATPase family</b>							
contig27074:1:762:2 similar to 26S protease regulatory subunit 4 (P48601) Evalue: 9e-105	59	2	8.3	253	0.92	0.35	0.49
contig23675:1:1205:2 similar to 26S protease regulatory subunit 4 (P48601) Evalue: 5e-168	82	2	6	401		0.15	
contig02305:1073:1710:3 similar to 26S protease regulatory subunit 4 (P48601) Evalue: 5e-111	292	5	32.7	211	1.49	0.92	0.99
contig25304:139:1407:1 similar to 26S protease regulatory subunit 8 (P62195) Evalue: 0.0	589	14 (12)	40	422	4.78	1.47	2.22
contig24424:125:1390:2 similar to 26S protease regulatory subunit 6B (P43686) Evalue: 0.0	693	15	46.1	421	1.53	1.6	2.16
contig24391:1:1070:3 similar to 26S protease regulatory subunit 7 (P35998) Evalue: 0.0	649	17 (13)	50.7	355	2.38	2.57	2.38
contig23398:197:431:2 similar to 26S protease regulatory subunit 7 (P35998) Evalue: 2e-11	140	3	43.6	78		2.14	0.96
contig08447:1:767:3 similar to 26S protease regulatory subunit S10B (P62333) Evalue: 2e-95	208	7 (4)	19.6	255	1.14	0.83	0.66
contig06140:61:324:1 similar to 26S protease regulatory subunit 6A (P17980) Evalue: 2e-23	91	3	26.1	88	1.4		
contig03769:1:516:1 similar to Probable 26S protease regulatory subunit S10B (O17071) Evalue: 2e-82	184	4	18.7	171	2.04	0.89	0.89
contig02055:1:827:3 similar to 26S protease regulatory subunit 6A (P17980) Evalue: 2e-144	441	14 (8)	42.9	275	1.8	1.91	2.11
contig00555:1:185:3 similar to 26S protease regulatory subunit 6A (P17980) Evalue: 1e-22	77	2	38.3	60	1.28		1.28
<b>others</b>							
contig18880:1:1138:2 similar to Transmembrane protease, serine 13 (Q5U405) Evalue: 7e-17	516	17 (8)	46.6	378	2	1.97	1.81
contig19551:47:1534:2 similar to Transmembrane protease, serine 6 (Q9DBI0) Evalue: 1e-40	146	3	9.7	495		0.25	
contig23430:649:975:1 similar to Transmembrane protease, serine 5 (Q9ER04) Evalue: 7e-17	317	12 (6)	59.3	108		6.69	4.3
contig23355:1:1824:1 similar to Ufm1-specific protease 2 (Q3B8N0) Evalue: 5e-68	132	3	6.1	607	0.21	0.15	0.1
contig25969:1:208:2 similar to Thiol protease aleurain (P05167) Evalue: 1e-10	123	2	33.8	68		2.01	2.22
contig18641:1:963:2 similar to Chymotrypsin-like protease CTRL-1 (P40313) Evalue: 3e-13	89	2	8.4	320	0.2		
contig21807:1:609:2 similar to Chymotrypsin-like protease CTRL-1 (P40313) Evalue: 5e-17	261	12 (5)	23.8	202		1.78	
contig21807:1:609:2 similar to Chymotrypsin-like protease CTRL-1 (P40313) Evalue: 5e-17	217	12 (4)	20.3	202			1.46
contig22297:1:1374:1 similar to Cysteine protease ATG4B (Q8BGE6) Evalue: 2e-89	93	2	8.1	457			0.13
contig19926:1:674:2 similar to CAAX prenyl protease 1 homolog (O75844) Evalue: 4e-54	76	1	6.7	224	0.67	0.26	0.41
contig12607:150:967:3 similar to Inactive testicular serine protease 1 (O70169) Evalue: 5e-14	375	10 (8)	36.4	272		1.33	1.09

## Supplementary

contig05601:147:692:3 similar to CAAX prenyl protease 1 homolog (Q80W54) Evalue: 3e-27	33	1	5.5	181	0.32	0.32	
contig03034:1:1882:2 similar to Lon protease homolog, mitochondrial (P36776) Evalue: 1e-159	147	2	5.3	627		0.09	
contig03013:169:999:1 similar to Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial (O88696) Evalue: 3e-78	51	1	8	276		0.22	
contig01597:127:471:1 similar to Astacin-like metalloprotease toxin (A0FKN6) Evalue: 4e-04	159	3	25.2	115		0.98	
contig00907:1:161:3 similar to Sentrin-specific protease 8 (Q9D2Z4) Evalue: 5e-05	29	1	11.5	52			1.12
<b>Ras-related proteins</b>							
contig28006:1:112:2 similar to Ras-related protein Rab-7a (P09527) Evalue: 8e-14	103	3 (2)	56.8	37	3.7	2.76	2.76
contig26611:146:582:2 similar to Ras-related protein Rab-1A (Q6NYB7) Evalue: 2e-75	292	11 (6)	44.1	145	2.01	2.41	3.56
contig25529:1:150:2 similar to Ras-related protein Rab-18 (Q5ZLG1) Evalue: 9e-16	100	2 (1)	30.6	49	1.3		1.73
contig24936:91:732:1 similar to Ras-related protein Rab-11A (Q5ZJN2) Evalue: 5e-100	421	11 (8)	55.4	213	4.9	4.88	3.63
contig24378:1:870:1 similar to Ras-related protein Rab-5B (Q5ZHW4) Evalue: 2e-80	434	13 (7)	33.6	289	1.52	2.06	1.84
contig24142:1:612:2 similar to Ras-related protein Rab-14 (Q5ZKU5) Evalue: 9e-84	333	7 (6)	38.9	203	1.47	1.57	1.03
contig23522:1:397:2 similar to Ras-related protein Rab-10 (Q5ZIT5) Evalue: 2e-47	408	11 (7)	60.3	131	2.95	3.52	4.18
contig23258:1:283:2 similar to Ras-related protein Rab-43 (Q8CG50) Evalue: 1e-08	68	1	15.1	93	0.77	0.66	0.66
contig22898:1:238:2 similar to Ras-related protein Rab-10 (Q5ZIT5) Evalue: 5e-17	146	4 (3)	30.4	79	1.65	3.3	2.56
contig22723:113:433:2 similar to Ras-related protein Rab-21 (Q17R06) Evalue: 5e-45	75	2	20.6	107		0.64	
contig22280:1:354:1 similar to Ras-related protein Rab-18 (Q5ZLG1) Evalue: 2e-51	182	5 (3)	34.7	118	1.77	1.25	0.99
contig21654:1:366:1 similar to Ras-related protein Rab-21 (P35282) Evalue: 2e-27	60	1	9.1	121		0.5	0.5
contig18817:13:674:1 similar to Ras-related protein Rab-43 (Q86YS6) Evalue: 6e-50	92	3 (2)	11.8	220	0.45	0.28	0.28
contig17820:1129:1686:1 similar to Ras-related protein Rap-1b (Q5RDM6) Evalue: 3e-82	374	7 (6)	36.8	185	3.51	1.58	1.51
contig12781:1:380:1 similar to Ras-related protein Rab-2A (Q90965) Evalue: 7e-38	65	2	16.7	126	0.52		
contig12673:1050:1649:3 similar to Ras-related protein R-Ras2 (P62071) Evalue: 5e-70	360	7 (5)	28.6	199		0.96	0.96
contig09118:750:1295:3 similar to Ras-related protein Rap-2c (Q9Y3L5) Evalue: 3e-64	72	2	10.5	181	0.35		
contig08919:1:468:2 similar to Ras-related protein Rab-2A (Q90965) Evalue: 2e-82	314	6	48.4	155	3.4	2.17	2.96
contig08277:139:639:1 similar to Ras-related protein Rab-6A (Q1KME6) Evalue: 4e-76	126	4 (3)	17.4	167		1.2	1.26
contig06795:1:925:2 similar to Ras-related protein Rab-3 (P25228) Evalue: 2e-104	274	5 (4)	18.9	307		0.43	0.4
contig05996:1:211:3 similar to Ras-related protein Rab-6.2 (Q22782) Evalue: 4e-31	74	2	34.8	69	1.52		
contig05524:2260:2838:3 similar to Ras-related protein Rac1 (P40792) Evalue: 2e-100	63	2	10.9	192	0.77		
contig04133:1:269:1 similar to Ras-related protein Rab-6A (Q5RAV6) Evalue: 3e-45	100	3 (2)	24.7	89	1.56		1.38
contig02806:1:365:2 similar to Ras-related protein Rab-1A (Q05974) Evalue: 2e-40	92	3 (2)	18.2	121	0.82		1.07
contig02785:1:266:3 similar to Ras-related protein Rab-35 (Q15286) Evalue: 8e-16	187	3	41.4	87	1.38	2.76	2.52
contig01793:1:763:2 similar to Ras-related protein Ral-a (P48555) Evalue: 2e-67	121	4 (3)	11.9	253	0.51	0.75	1.17
contig00683:1:486:1 similar to Ras-related protein Rab-7a (Q3T0F5) Evalue: 1e-71	516	14 (8)	68.3	161	5.79	4.42	4.24
<b>Transferases</b>							
contig01553:1:2297:3 similar to Glycogen [starch] synthase, muscle (A7MB78) Evalue: 0.0	393	10 (9)	12	764		0.59	0.66
contig09564:202:1650:1 similar to Acetolactate synthase-like protein (Q6NV04) Evalue: 8e-117	376	5	19.9	483		0.35	0.35
contig01045:279:2180:3 similar to Gamma-glutamyltranspeptidase 1 (P20735) Evalue: 4e-84	129	3	5.1	633	0.35	0.3	0.25
contig01620:1:1397:3 similar to Gamma-glutamyltranspeptidase 1 (P07314) Evalue: 4e-88	1298	51 (19)	49.5	465		7.52	6.99
contig02904:228:610:3  histone-lysine N-methyltransferase ASHR1 [Zea mays] (GI_195636164) Evalue: 7e-05	70	2	19.7	127	1.04	0.98	0.98
contig28066:1:242:3 similar to 3-alpha-hydroxysteroid sulfotransferase (P50234) Evalue: 6e-12	128	2 (1)	27.5	80			1.21
contig27860:1:1412:3 similar to Putative polypeptide N-acetylgalactosaminyltransferase 10 (O45947) Evalue: 1e-152	111	3	8.7	469		0.19	0.12

## Supplementary

contig26570:177:1199:3 similar to Sulfotransferase 1A3/1A4 (P50224) Evalue: 3e-40	122	3 (2)	11.5	340		0.27	0.17
contig27801:7:140:1 similar to Aspartate aminotransferase, mitochondrial (Q28F67) Evalue: 3e-11	104	2	52.3	44	2.07		2.07
contig27598:1:848:3 similar to Glycine N-acyltransferase-like protein 3 (Q5SZD4) Evalue: 6e-09	126	3	12.8	281		0.33	0.21
contig25975:14:364:2 similar to Probable glucosamine 6-phosphate N-acetyltransferase (Q9VAI0) Evalue: 3e-18	229	4	37.9	116	1.2	0.25	1.44
contig26751:165:2444:3 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A (Q5RCE2) Evalue: 0.0	306	7	10.7	759	0.36	0.41	0.58
contig26745:1:734:3 similar to Phosphoserine aminotransferase (Q99K85) Evalue: 7e-83	95	2	9.1	243	1.59	0.25	0.88
contig26031:1:344:3 similar to Aspartate aminotransferase, mitochondrial (Q4R559) Evalue: 4e-38	152	4 (3)	28.3	113	1.05	1.32	1.05
contig25773:1:614:3 similar to GTP:AMP phosphotransferase mitochondrial (Q9WTP7) Evalue: 7e-42	537	20 (11)	64.5	203	2.07	6.13	5.56
contig25756:1:500:2 similar to rRNA 2--O-methyltransferase fibrillarin (Q9W1V3) Evalue: 4e-69	93	3 (2)	18.7	166	0.97	0.4	1.51
contig25702:1:339:1 similar to Aspartate aminotransferase, mitochondrial (P00508) Evalue: 1e-48	168	5 (3)	31.2	112	1.04	1.31	2.82
contig25492:1062:1391:1 similar to Mannose-1-phosphate guanyltransferase beta (Q9Y5P6) Evalue: 2e-21	74	2	15.6	109		1.32	1.43
contig25359:2854:3612:2 similar to Uncharacterized methyltransferase GK2543 (Q5KWV8) Evalue: 5e-05	619	12 (8)	42.1	252	0.24	2.1	2.83
contig25295:1:539:3 similar to 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase-like protein (A4D126) Evalue: 1e-30	233	6 (4)	36	178			0.87
contig25105:2021:3415:1 similar to Aminoglycoside phosphotransferase domain-containing protein 1 (Q6PB06) Evalue: 2e-23	268	6 (5)	17.2	464		0.44	0.13
contig25082:1:895:2 similar to Sulfotransferase 1C4 (O75897) Evalue: 9e-40	99	3	10.4	297	0.44	0.31	
contig24677:1:1007:3 similar to Sulfotransferase 1C4 (O75897) Evalue: 2e-36	77	2	7.5	334		0.18	0.51
contig24237:17:964:2 similar to Sulfotransferase 1A3/1A4 (P50224) Evalue: 2e-45	128	3	11.1	315	0.3		
contig24234:1:1057:2 similar to Sulfotransferase 1C4 (O75897) Evalue: 5e-36	142	5 (4)	17.4	351		0.45	0.54
contig19694:48:1100:3 similar to Sulfotransferase 1C2 (Q9D939) Evalue: 2e-44	627	16 (10)	45.7	350	0.17	2.39	2.52
contig16810:1:694:2 similar to Sulfotransferase 1A1 (Q29476) Evalue: 6e-13	72	2	12.6	230	0.27		
contig24953:69:1148:3 similar to Choline-phosphate cytidyltransferase B (Q811Q9) Evalue: 3e-85	231	4	14.8	359	0.26	0.36	0.26
contig24555:1:521:3 similar to Dolichol-phosphate mannosyltransferase (O70152) Evalue: 1e-74	148	5 (4)	28.5	172	1.61	1.39	1.07
contig24534:74:284:2 similar to Probable dolichol-phosphate mannosyltransferase (Q9VIU7) Evalue: 4e-19	212	4 (3)	70	70	1.06	3.23	
contig24466:1:1154:1 similar to Alanine--glyoxylate aminotransferase 2-like (Q9VU95) Evalue: 2e-110	85	2	7.3	384			0.16
contig24354:1:1042:2 similar to Glutaminy-peptide cyclotransferase (Q16769) Evalue: 5e-61	120	3	8.6	347		0.27	0.17
contig24195:1406:3009:2 similar to Polypeptide N-acetylgalactosaminyltransferase 13 (Q8IUC8) Evalue: 2e-174	265	5	12.7	534		0.3	0.11
contig23775:167:587:2 similar to Sulfotransferase family cytosolic 1B member 1 (Q8JG30) Evalue: 8e-10	62	1	10.7	140			0.42
contig23533:1239:1931:1 similar to N-acetyltransferase 9-like protein (Q9V9V9) Evalue: 2e-28	190	5	25.7	230	0.61	0.94	0.43
contig23468:180:962:3 similar to Mannose-1-phosphate guanyltransferase beta (Q6DBU5) Evalue: 2e-95	360	5	31.4	261	1.42	1.35	1.61
contig23305:1:808:3 similar to Protein arginine N-methyltransferase 1 (Q99873) Evalue: 5e-92	358	8 (6)	28.7	268	3.87	1.03	1.24
contig23192:88:1508:1 similar to Alanine aminotransferase 2 (Q8TD30) Evalue: 3e-172	1127	28 (20)	51.2	473	0.83	2.93	3.57
contig22964:1:1955:3 similar to Carnitine O-palmitoyltransferase 1, muscle isoform (Q924X2) Evalue: 0.0	206	4	8	650	0.64	0.31	0.28
contig22510:1:1960:2 similar to Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] (P14742) Evalue: 0.0	873	20 (17)	37.4	652	1.2	1.88	1.19
contig22423:50:1672:2 similar to Beta-1,4-mannosyltransferase egh (O01346) Evalue: 7e-74	475	10 (8)	21.3	540		3.75	2.7
contig22187:1:180:2 similar to Aspartate aminotransferase, mitochondrial (P00505) Evalue: 2e-19	47	2 (1)	16.9	59		1.59	
contig22014:1:799:2 similar to Serine hydroxymethyltransferase, cytosolic (Q5E9P9) Evalue: 5e-83	306	9 (7)	27.5	265	0.72	1.47	1.25
contig21997:160:1444:1 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 (Q4R4T0) Evalue: 1e-111	485	14 (10)	25	428	1.67	3.48	2.58
contig21689:47:381:2 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit dad-1 (P52872) Evalue: 1e-40	55	1	8.1	111	0.54	0.81	0.81
contig11404:1768:2695:2 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	458	15 (9)	44.8	308	2.92	4.92	4



## Supplementary

(Q6GNR9) Evalue: 1e-87											
contig09625:1:2318:3 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A (Q5RCE2) Evalue: 0.0	487	13 (10)	16.1	771	0.44	1.42	0.87				
contig11294:1:233:1 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit (Q24319) Evalue: 1e-20	205	4 (3)	66.2	77	4.95	6.21	6.03				
contig21748:56:622:2 similar to Nicotinamide mononucleotide adenylyltransferase 3 (Q99JR6) Evalue: 4e-39	51	2 (1)	5.3	188	0.53	0.3	0.48				
contig21724:131:886:2 similar to Hypoxanthine-guanine phosphoribosyltransferase (Q3SZ18) Evalue: 3e-51	100	2	10.4	251	0.25	0.37	0.24				
contig21534:1:845:3 similar to Acetyl-CoA acetyltransferase, mitochondrial (Q5BKN8) Evalue: 2e-97	381	8 (6)	28.6	280	1.1	1.45	1.45				
contig21274:1:742:2 similar to tRNA (uracil-5-)-methyltransferase homolog A (Q8IZ69) Evalue: 7e-19	94	2	13.8	247	0.25						
contig21159:1:690:1 similar to N(6)-adenine-specific DNA methyltransferase 2 (Q9CY45) Evalue: 1e-34	81	2	13.5	229		0.27	0.27				
contig21053:1:646:3 similar to Serine hydroxymethyltransferase, cytosolic (P34896) Evalue: 7e-82	530	12 (7)	54.2	214	2.25	3.72	4.52				
contig20721:1:488:3 similar to Diamine acetyltransferase 2 (Q7PCJ9) Evalue: 3e-22	120	2	14.9	161		0.4					
contig20457:1:1803:1 similar to Carnitine O-acetyltransferase (Q704S8) Evalue: 3e-121	180	4	7.5	600	0.1	0.26	0.21				
contig07876:93:2093:3 similar to Carnitine O-palmitoyltransferase 2, mitochondrial (Q6P4X5) Evalue: 0.0	417	10 (8)	15.5	666	0.23	0.76	0.5				
contig20416:148:1380:1 similar to Beta-1,3-galactosyltransferase 5 (Q9N294) Evalue: 2e-19	265	6 (5)	13.4	410		0.4	0.22				
contig20155:234:665:3 similar to Phosphoserine aminotransferase (P10658) Evalue: 6e-31	156	5 (2)	18.8	144	2.39	1.25	0.48				
contig20082:5:163:2 similar to Protein-L-isoaspartate(D-aspartate) O-methyltransferase (Q5RA89) Evalue: 1e-05	69	1	32.7	52		1.26	2.27				
contig19975:182:1018:2 similar to Nicotinamide N-methyltransferase (Q06AV1) Evalue: 2e-26	347	8 (6)	29.5	278		0.8	0.8				
contig19487:96:1007:3 similar to Methionine adenosyltransferase 2 subunit beta (Q566L8) Evalue: 4e-85	235	4	16.2	303	0.33	0.56	0.33				
contig19481:1:774:3 similar to tRNA (uracil-5-)-methyltransferase homolog A (Q8IZ69) Evalue: 2e-51	168	4	18.7	257	0.54						
contig19378:129:1640:3 similar to UTP--glucose-1-phosphate uridylyltransferase (P79303) Evalue: 0.0	491	10	20.3	503		0.75	1.19				
contig18477:1:119:1 similar to Aspartate aminotransferase, mitochondrial (Q28F67) Evalue: 1e-13	127	2	38.5	39	6.28	5.04	5.78				
contig18095:1:491:3 similar to Aspartate aminotransferase, mitochondrial (P08907) Evalue: 6e-64	421	9 (6)	52.1	163	1.54	3.36	2.77				
contig03322:1:603:2 similar to Aspartate aminotransferase, mitochondrial (P00507) Evalue: 2e-37	330	8 (6)	33	200	1.33	1.68	2.55				
contig01829:141:480:3 similar to Aspartate aminotransferase, mitochondrial (P00507) Evalue: 3e-31	219	7 (4)	38.1	113	1.08	2.66	1.93				
contig25455:1:1318:2 similar to Ornithine aminotransferase, mitochondrial (Q9VW26) Evalue: 1e-160	434	8 (7)	23.1	438		0.58	0.39				
contig18295:82:424:1 similar to Ethanolamine-phosphate cytidylyltransferase (Q99447) Evalue: 9e-46	201	4 (3)	27.2	114		1.81	1.02				
contig10246:1:782:3 similar to Ethanolamine-phosphate cytidylyltransferase (Q5EA75) Evalue: 3e-68	316	6	20.5	259	0.38	1.4	1.47				
contig17176:403:610:2 similar to Protein arginine N-methyltransferase 1 (Q99873) Evalue: 3e-14	43	3 (1)	22.1	68	1.09		1.09				
contig10999:1:909:1 similar to Histone-lysine N-methyltransferase ASHR1 (Q7XJS0) Evalue: 4e-14	320	8 (7)	25.8	302	0.44	1.62	1.71				
contig09663:177:1946:3 similar to Histone-arginine methyltransferase CARMER (Q29B63) Evalue: 1e-151	67	2	2.4	589	0.42	0.15	0.1				
contig16741:1187:1707:3 similar to Formimidoyltransferase-cyclodeaminase (Q9YH58) Evalue: 4e-32	289	7 (4)	38.4	172	1.26	1.1	1.65				
contig11987:1:599:3 similar to Adenine phosphoribosyltransferase (O42842) Evalue: 3e-34	351	4	34.8	198	0.78						
contig10401:1:1079:3 similar to Protein-tyrosine sulfotransferase (Q9VYB7) Evalue: 4e-107	137	4 (3)	10.1	358	0.26						
contig11582:495:1688:3 similar to UDP-glucose:glycoprotein glucosyltransferase (Q09332) Evalue: 9e-135	247	7 (6)	20.1	398	0.15	0.7	0.32				
contig08793:1:2293:2 similar to Protein arginine N-methyltransferase 2 (Q5ZIB9) Evalue: 9e-130	68	2	2.5	763	0.08						
contig08684:1:921:1 similar to Acetyl-CoA acetyltransferase, cytosolic (Q9BWD1) Evalue: 1e-94	309	7 (6)	32	306		0.99	0.48				
contig07146:162:576:3 similar to Acetyl-CoA acetyltransferase, mitochondrial (Q29RZ0) Evalue: 2e-35	208	4	44.9	138	0.53	1.33	0.53				
contig06687:139:795:1 similar to N-terminal acetyltransferase B complex catalytic subunit NAT5 (Q58ED9) Evalue: 1e-43	162	3	19.7	218	0.73	0.45	0.45				
contig03086:118:795:1 similar to N-terminal acetyltransferase complex ARD1 subunit homolog B (Q9BSU3) Evalue: 8e-67	135	3	18.2	225	0.8	0.68	0.38				
contig07994:1:373:2 similar to Alanine--glyoxylate aminotransferase 2, mitochondrial (Q5RFA3) Evalue: 7e-34	85	3 (2)	20.3	123		0.55	0.55				
contig07594:1:630:1 similar to GDP-fucose protein O-fucosyltransferase 1 (Q91ZW2) Evalue: 1e-31	83	2	12.4	209	0.3						

## Supplementary

contig06618:1:1179:1 similar to Alanine--glyoxylate aminotransferase 2, mitochondrial (Q17QF0) Evalue: 2e-120	253	7 (6)	25.7	393		0.61	0.78
contig06077:1:449:3 similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 (P04844) Evalue: 3e-14	37	1	4.1	148	0.4		
contig05829:1:1288:2 similar to Aminomethyltransferase, mitochondrial (Q8CFA2) Evalue: 1e-99	155	3	9.1	428		0.29	0.47
contig05621:296:1522:2 similar to Branched-chain-amino-acid aminotransferase, cytosolic (P24288) Evalue: 6e-120	332	7	24	408	0.38	0.94	0.47
contig05174:1:1157:2 similar to Leucine carboxyl methyltransferase 1 (Q9UIC8) Evalue: 1e-67	131	2	7	385		0.16	0.16
contig04283:219:2267:3 similar to N-acetylgalactosaminyltransferase 7 (Q8MV48) Evalue: 6e-180	139	2	3.4	682		0.08	0.13
contig04037:108:1667:3 similar to Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial (B2GV06) Evalue: 0.0	381	8 (7)	15.4	519		0.48	0.72
contig03405:179:1102:2 similar to 3-demethylubiquinone-9 3-methyltransferase (Q1QEI9) Evalue: 5e-30	516	18 (9)	31.9	307	3.46	2.03	2.48
contig03247:158:1759:2 similar to Lysophospholipid acyltransferase 2 (Q6ZWT7) Evalue: 2e-82	89	2	4.7	533		0.16	
contig02775:57:1240:3 similar to Formimidoyltransferase-cyclodeaminase (Q91XD4) Evalue: 5e-124	451	9 (7)	33.2	394	1.19	1.3	1.14
contig02395:368:1717:2 similar to Glycerol-3-phosphate acyltransferase 3-like (A3KGT9) Evalue: 2e-130	55	1	2.7	449		0.12	1.19
contig02277:83:933:2 similar to Amine sulfotransferase (O46640) Evalue: 1e-25	59	2	6.4	283			0.22
contig02189:135:1202:3 similar to Galactose-1-phosphate uridylyltransferase (Q03249) Evalue: 8e-112	70	2	6.5	355		0.16	0.16
contig02151:2470:2607:1 similar to rRNA/tRNA 2--O-methyltransferase fibrillar-like protein 1 (A6NHQ2) Evalue: 1e-10	119	2	53.3	45	3.88	6.71	4.77
contig02139:1:690:1 similar to Diacylglycerol O-acyltransferase 2 (Q96PD7) Evalue: 3e-63	213	5	31	229	0.75	1.1	0.57
contig01775:17:1288:2 similar to Uncharacterized UDP-glucosyltransferase yojK (O31853) Evalue: 5e-19	153	4 (3)	8	423		0.22	0.14
contig01642:1:452:3 similar to UDP-glucose:glycoprotein glucosyltransferase 1 (Q6P5E4) Evalue: 1e-49	93	2	27.5	149		0.43	
contig01106:1:301:2 similar to Alanine--glyoxylate aminotransferase 2-like (Q9VU95) Evalue: 6e-23	84	2	27.3	99		0.69	
contig00745:1:520:1 similar to Protein-L-isoaspartate(D-aspartate) O-methyltransferase (Q4R5H0) Evalue: 1e-35	37	1	5.8	173		0.34	
contig00602:234:2048:3 similar to Nicotinate phosphoribosyltransferase (Q9VQX4) Evalue: 0.0	125	3	5.8	604	0.21	0.1	0.21
contig00405:208:1710:1 similar to 4-aminobutyrate aminotransferase, mitochondrial (P50554) Evalue: 2e-159	1009	26 (18)	50.2	500		2.87	3.2
<b>Synthases</b>							
contig08799:1:1630:2 similar to Citrate synthase, mitochondrial (Q28DK1) Evalue: 0.0	1285	69 (22)	50.9	542	2.39	7.86	3.91
contig06053:1144:1611:1 similar to ATP-citrate synthase (Q32PF2) Evalue: 1e-65	144	4 (3)	21.9	155		0.87	0.6
contig06053:1:967:2 similar to ATP-citrate synthase (Q91V92) Evalue: 1e-128	393	7 (6)	27.7	321		1.21	0.84
contig05151:1:305:3 similar to ATP-citrate synthase (P53396) Evalue: 2e-18	115	2	21.8	101		1.36	0.6
contig00063:1:1523:3 similar to Trimethylguanosine synthase (P85107) Evalue: 6e-59	124	2	4.7	507	0.12		0.12
contig19884:167:2023:2 similar to Glucose 1,6-bisphosphate synthase (Q8CAA7) Evalue: 0.0	390	7	16.5	618	0.1	0.43	0.38
contig25408:1:1044:1 similar to Deoxyhypusine synthase-like protein (B2IZP9) Evalue: 1e-11	158	4 (3)	11.2	347	0.17	0.38	0.62
contig24825:1:1985:3 similar to CTP synthase 1 (Q6PEI7) Evalue: 0.0	273	6	10	660	0.3		
contig21748:619:1418:1 similar to Uroporphyrinogen-III synthase (P51163) Evalue: 5e-32	136	4 (3)	13.5	266	0.24	0.62	0.48
contig17969:1:910:2 similar to Spermidine synthase (Q64674) Evalue: 2e-83	61	2	9.3	302		0.2	0.2
contig14740:385:519:1 similar to Putative pseudouridine synthase YOR243C (Q08647) Evalue: 2e-04	51	2	13.3	45		0.75	
contig11165:202:1374:1 similar to Cystathionine beta-synthase (P32232) Evalue: 1e-132	376	7	29.7	390	0.55	0.67	1.02
contig06876:2455:3520:2 similar to Probable deoxyhypusine synthase (Q9VSF4) Evalue: 3e-112	642	13 (9)	37	354	1.78	1.54	1.28
contig06537:1:1309:2 similar to Threonine synthase-like 2 (Q86YJ6) Evalue: 2e-105	175	3	8.5	435			0.21
contig03867:1:1472:3 similar to C-1-tetrahydrofolate synthase, cytoplasmic (Q5R8P0) Evalue: 0.0	557	9	27	489	0.19	0.71	0.61
contig00777:101:1024:2 similar to C-1-tetrahydrofolate synthase, cytoplasmic (P27653) Evalue: 1e-93	196	4	16	307	0.46		
contig01946:1:1366:2 similar to Probable methionine synthase (Q09582) Evalue: 1e-141	70	2	6.6	455		0.19	0.13
contig27714:1:1436:3 similar to Glutathione synthetase (P48637) Evalue: 1e-75	488	10	26.6	477	0.34	0.8	0.34
contig25813:1:416:2 similar to ATP synthase subunit O, mitochondrial (Q2EN81) Evalue: 8e-25	355	14 (7)	52.2	138	2.09	5.79	3.61

contig23516:1:322:2 similar to ATP synthase subunit e, mitochondrial (Q00361) Evalue: 4e-05	89	4 (2)	35.8	106	2.48	0.64	0.64
contig23461:107:1792:2 similar to ATP synthase subunit alpha (A0LDA2) Evalue: 0.0	1774	76 (29)	51.5	561	6.84	16.86	13.84
contig22061:1:563:1 similar to ATP synthase subunit b, mitochondrial (Q94516) Evalue: 5e-13	348	11 (7)	62	187	1.77	4.91	3.97
contig20012:1:298:2 similar to ATP synthase subunit b, mitochondrial (Q94516) Evalue: 5e-26	303	7 (6)	45.9	98	1.16	6.26	5.2
contig04301:1:1559:3 similar to ATP synthase subunit beta (Q39Q56) Evalue: 0.0	1992	120 (26)	75.1	518	13.17	34.93	39.26
contig19588:68:937:2 similar to ATP synthase subunit gamma, mitochondrial (P05631) Evalue: 1e-97	599	24 (12)	58.1	289	2.21	5.75	4.57
contig19529:448:736:1 similar to ATP synthase subunit d, mitochondrial (Q24251) Evalue: 6e-25	405	13 (7)	56.2	96	4.19	8.62	11.41
contig17562:1:409:1 similar to Putative ATP synthase subunit f, mitochondrial (Q9W141) Evalue: 8e-19	40	1	7.4	136	0.92	0.84	
contig04841:105:284:3 similar to ATP synthase subunit epsilon, mitochondrial (P05632) Evalue: 3e-09	48	1	16.9	59	3.26	1.08	
contig22780:74:272:2 similar to ATP synthase subunit g, mitochondrial (Q5RFH0) Evalue: 6e-10	86	5 (2)	30.3	66	2.4		
contig20250:1:631:2 similar to ATP synthase subunit delta, mitochondrial (Q9D3D9) Evalue: 6e-40	81	1	6.7	209	0.3		
contig10432:16:481:1 similar to C-1-tetrahydrofolate synthase, cytoplasmic (P11586) Evalue: 4e-52	151	3	29.7	155	0.44	0.74	0.44
<b>Ribosomal proteins/Translation</b>							
contig27403:1:345:2 similar to 40S ribosomal protein S16 (Q98TR7) Evalue: 3e-42	108	2	17.5	114	0.59		
contig19549:1:1193:3 similar to 28S ribosomal protein S9, mitochondrial (P82933) Evalue: 6e-77	120	2	5.8	396	0.32	0.15	0.15
contig17866:98:556:2 similar to 40S ribosomal protein S16 (Q3T0X6) Evalue: 1e-63	254	10 (7)	46.7	152	8.08	3.63	2.13
contig27302:1:601:1 similar to 40S ribosomal protein S8 (Q8WQI5) Evalue: 3e-58	202	3	24	200	0.51		0.51
contig17747:86:712:2 similar to 40S ribosomal protein S8 (Q8WQI5) Evalue: 1e-84	532	17 (9)	49	208	5.9	7.62	5.07
contig27248:112:418:1 similar to 60S ribosomal protein L17 (Q5UAS2) Evalue: 8e-41	326	21 (6)	46.1	102	5.84	11	5.4
contig18517:1:173:3 similar to 60S ribosomal protein L17 (P24049) Evalue: 3e-21	162	5 (2)	59.6	57	8.71	6.57	7.87
contig05245:59:874:2 similar to 40S ribosomal protein S0 (A8N7C6) Evalue: 3e-88	246	5	25.8	271	0.34	0.69	0.87
contig05022:19:486:1 similar to 60S ribosomal protein L17 (Q6CBS7) Evalue: 1e-46	70	2	11	155	0.41		
contig27230:99:680:3 similar to 40S ribosomal protein S7 (A6H769) Evalue: 1e-60	439	15 (6)	46.1	193	4.05	3.88	3.8
contig12391:45:293:3 similar to 40S ribosomal protein S7 (Q9VA91) Evalue: 5e-07	95	2	32.9	82		0.89	0.89
contig27073:1:312:1 similar to 60S ribosomal protein L44 (P48166) Evalue: 8e-20	130	11 (3)	17.5	103	1.05		
contig01191:1:298:2 similar to 40S ribosomal protein S3 (Q90YS2) Evalue: 2e-32	343	20 (6)	81.6	98	11.11	31.11	24.94
contig26860:133:289:1 similar to 40S ribosomal protein S3 (Q90YS2) Evalue: 3e-13	226	10 (4)	57.7	52	9.52	9.77	22.28
contig17608:1:572:1 similar to 40S ribosomal protein S3 (Q3T169) Evalue: 7e-71	187	4	23.2	190	0.32	1.62	1.04
contig26558:1:1367:1 similar to 40S ribosomal protein S6 (P39017) Evalue: 2e-92	292	9 (7)	17.4	455	2.07	2.35	1.55
contig22930:17:653:2 similar to 40S ribosomal protein S6-B (Q9C0Z7) Evalue: 2e-65	63	1	6.1	212			0.28
contig26352:1:274:2 similar to 60S ribosomal protein L26 (Q95WA0) Evalue: 4e-25	223	6 (4)	45.6	90	7.5	5.33	7.78
contig05681:124:251:1 similar to 60S ribosomal protein L26 (P61256) Evalue: 7e-12	102	4 (3)	64.3	42	26.47	6.21	8.39
contig26340:1:429:1 similar to 40S ribosomal protein S14 (P19951) Evalue: 1e-51	101	2	17.6	142	0.74		
contig05614:107:562:2 similar to 40S ribosomal protein S14 (P48150) Evalue: 2e-57	154	4 (3)	29.8	151	7.18	1.82	2.84
contig26212:1:137:3 similar to 40S ribosomal protein S27-like protein (Q3T0B7) Evalue: 1e-17	32	2 (1)	28.9	45	3.78		
contig26208:1:1064:3 similar to 40S ribosomal protein S28 (Q6PBK3) Evalue: 1e-15	176	4 (3)	8.8	353	0.43		
contig26071:60:440:3 similar to 60S ribosomal protein L31 (Q9GP16) Evalue: 5e-43	36	1	7.1	126	3.43	0.46	0.74
contig26014:1:184:2 similar to 40S ribosomal protein S23 (Q8T3U2) Evalue: 1e-21	76	2 (1)	18.3	60	1.59		
contig17772:1:166:2 similar to 40S ribosomal protein S23 (P62298) Evalue: 2e-23	152	3 (2)	42.6	54	10.83	5.86	1.2
contig01239:1:484:2 similar to 40S ribosomal protein S23 (Q873W8) Evalue: 6e-60	54	2	13.8	160	0.41		
contig25753:1:331:1 similar to 60S ribosomal protein L11 (P62914) Evalue: 3e-52	162	6 (3)	26.4	110	2.08	2.69	1.59

## Supplementary

contig23713:1:197:3 similar to 60S ribosomal protein L11 (P62914) Evalue: 1e-22	115	9 (2)	23.4	64	2.36	4.02	3.54
contig04764:1:263:3 similar to 60S ribosomal protein L11 (P62914) Evalue: 7e-40	70	1	16.1	87	1.05		0.83
contig25338:1:169:1344:2 similar to 60S ribosomal protein L15 (Q5EAD6) Evalue: 2e-21	39	1	12.1	58	1.2	1.44	1.92
contig25303:1:706:2 similar to 60S ribosomal protein L7 (Q7SBD5) Evalue: 1e-75	139	3	14.5	234			0.53
contig08851:1:613:2 similar to 60S ribosomal protein L7 (O01802) Evalue: 4e-82	577	21 (14)	62.6	203	39.62	13.27	8.23
contig24916:99:839:3 similar to 60S ribosomal protein L23a (P62750) Evalue: 5e-45	260	7 (5)	24	246	3.7	2.45	2.38
contig24375:105:560:3 similar to 40S ribosomal protein S13 (P47772) Evalue: 5e-69	307	11 (5)	31.1	151	4.14		0.72
contig00299:106:981:1 similar to 28S ribosomal protein S15, mitochondrial (Q8WTC1) Evalue: 6e-18	91	2	6.9	291	0.2		
contig24148:1:277:2 similar to 40S ribosomal protein S25 (Q8ISN9) Evalue: 7e-24	71	3 (2)	28.6	91	13.5	1.38	
contig24100:1:605:3 similar to 60S ribosomal protein L14 (A1XQU3) Evalue: 5e-37	309	13 (6)	28.5	200	2.79	2.81	3.64
contig12927:1:360:1 similar to 60S ribosomal protein L14 (Q63507) Evalue: 2e-18	71	2	9.2	119	0.82		
contig23875:2066:2840:2 similar to 40S ribosomal protein S2 (Q90YS3) Evalue: 6e-97	518	21 (12)	48.6	257	3.01	7.69	7.25
contig22542:72:842:3 similar to 40S ribosomal protein S2 (P25444) Evalue: 1e-87	127	3	14.5	256	0.25	0.4	0.75
contig23806:97:584:1 similar to 60S ribosomal protein L18a (Q3T003) Evalue: 2e-68	343	14 (7)	33.3	162	4.26	3.59	5.45
contig28253:123:575:3 similar to 60S ribosomal protein L20 (P05732) Evalue: 6e-54	102	2	13.9	151	0.6		
contig23726:1:650:3 similar to 40S ribosomal protein S19 (Q9CZX8) Evalue: 1e-45	68	2	8.8	215	5.97	0.57	1.17
contig23713:377:826:2 similar to 60S ribosomal protein L27a (Q56K03) Evalue: 1e-47	168	5 (3)	14.1	149	4.34	0.92	1.06
contig20075:1:909:1 similar to 39S ribosomal protein L15, mitochondrial (Q0VC21) Evalue: 9e-71	59	2	5.3	302		0.09	
contig23494:325:819:1 similar to 40S ribosomal protein S10b (Q9VWG3) Evalue: 9e-38	388	21 (6)	40.9	164	9.58	8.4	7.56
contig23442:1:381:1 similar to 60S ribosomal protein L21 (Q6QN05) Evalue: 3e-39	176	7 (4)	31.7	126	2.96	2.06	2.5
contig18418:431:532:2 similar to 60S ribosomal protein L21 (A5JSS2) Evalue: 5e-06	28	1	35.3	34		1.82	
contig23397:100:410:1 similar to 40S ribosomal protein S26 (P27085) Evalue: 1e-47	89	1	14.6	103	1.21	0.56	
contig23322:1:889:2 similar to 60S ribosomal protein L4 (P49165) Evalue: 8e-99	540	14 (11)	44.3	296	7.9	6.51	4.92
contig02841:892:1129:1 similar to 60S ribosomal protein L4 (Q9XF97) Evalue: 7e-06	93	3 (2)	34.2	79			1.65
contig23318:1:502:2 similar to 40S ribosomal protein S12 (P84175) Evalue: 1e-44	118	4 (3)	18.7	166	9.99		1.12
contig22983:1:456:1 similar to 60S ribosomal protein L10 (O61231) Evalue: 1e-46	287	8 (6)	41.1	151	5.52	3.32	2.62
contig08160:1:306:3 similar to 60S ribosomal protein L10 (O61231) Evalue: 5e-27	120	3 (2)	34.7	101	1.61	2.19	1.9
contig06621:1:630:1 similar to 60S ribosomal protein L10-B (Q9P769) Evalue: 2e-81	90	3 (2)	7.1	210	1.1	0.71	0.9
contig22602:1:375:1 similar to Probable 60S ribosomal protein L37-A (Q9VXX8) Evalue: 1e-28	122	3	20.2	124	1.38		
contig22161:1:143:1 similar to 60S ribosomal protein L18 (Q6F442) Evalue: 4e-15	76	6 (2)	36.2	47	3.41		
contig19752:1:370:2 similar to 60S ribosomal protein L18 (Q4PM04) Evalue: 3e-41	306	13 (7)	45.9	122	5.77	7.52	4.7
contig13341:1:550:2 similar to 60S ribosomal protein L18-B (Q8TFH1) Evalue: 4e-56	169	6 (3)	20.3	182	0.74	1.52	1.16
contig22152:1:228:1 similar to 60S ribosomal protein L23 (P48159) Evalue: 7e-35	50	1	10.7	75	11.15	0.84	
contig00169:92:286:2 similar to 60S ribosomal protein L23 (P48159) Evalue: 4e-19	100	2 (1)	30.8	65	3.26		
contig22127:1:337:1 similar to 60S ribosomal protein L28 (P46779) Evalue: 2e-11	109	3 (2)	19.6	112	7.51	0.89	1.9
contig21585:1:492:1 similar to 40S ribosomal protein S5 (Q5E988) Evalue: 5e-88	468	18 (8)	39.3	163	1.92	6.41	4.9
contig18213:1:570:1 similar to 40S ribosomal protein S5 (Q7RVI1) Evalue: 8e-84	181	3	26.5	189		0.56	0.56
contig15336:1:254:3 similar to 40S ribosomal protein S5 (P49041) Evalue: 7e-27	79	1	18.1	83		1.48	
contig21510:1907:2014:1 similar to 40S ribosomal protein S30 (P62861) Evalue: 3e-05	226	7 (4)	77.1	35	43.57		
contig21336:1:487:2 similar to 60S ribosomal protein L35a (P02434) Evalue: 1e-37	111	6 (3)	18.6	161	6.71	2.13	1.24
contig21329:143:490:2 similar to 60S ribosomal protein L34 (Q9NB34) Evalue: 2e-30	186	7 (5)	28.7	115	2.33		

## Supplementary

contig21008:1:747:1 similar to 60S ribosomal protein L10a (Q963B6) Evalue: 1e-88	397	15 (6)	35.5	248	3.08	3.12	4.72
contig20801:92:886:2 similar to 40S ribosomal protein S3a (Q642T2) Evalue: 3e-94	705	32 (13)	51.1	264	10.82	14.21	17.04
contig04140:1:756:1 similar to 40S ribosomal protein S1 (A8NX92) Evalue: 7e-83	96	2	7.2	251	0.35	0.24	0.39
contig20627:1:208:2 similar to 60S ribosomal protein L9 (O02376) Evalue: 1e-11	151	6 (2)	40.6	69	6.79	6.31	6.55
contig04721:1:325:3 similar to 60S ribosomal protein L9 (Q963B7) Evalue: 1e-42	239	6 (3)	49.5	107	2.37	4	7.18
contig03838:1:268:2 similar to 60S ribosomal protein L9 (O02376) Evalue: 8e-21	120	3	39.3	89		1.47	1.18
contig03838:1:268:2 similar to 60S ribosomal protein L9 (O02376) Evalue: 8e-21	89	3 (2)	24.7	89			
contig20578:93:423:3 similar to 60S ribosomal protein L12 (Q6QMZ7) Evalue: 1e-48	204	9 (4)	43.6	110	9.63	3.62	4.62
contig19306:1:505:2 similar to 60S ribosomal protein L12 (O75000) Evalue: 6e-44	109	4 (2)	12.6	167	0.41		
contig16401:1:127:2 similar to 60S ribosomal protein L12 (P61866) Evalue: 3e-08	129	3 (2)	85.4	41	15.57	5.77	3.91
contig20456:1:386:1 similar to 40S ribosomal protein S9 (P29314) Evalue: 4e-63	479	15 (10)	48.4	128	11.6	10.22	11.09
contig17012:76:216:1 similar to 40S ribosomal protein S9 (P55935) Evalue: 1e-15	140	5 (4)	36.2	47	3.93	8.26	5.57
contig20321:1:864:1 similar to 60S acidic ribosomal protein P0 (Q9U3U0) Evalue: 8e-85	1129	51 (18)	76.3	287	17.51	27.44	31.26
contig09366:123:233:3 similar to 60S acidic ribosomal protein P0 (Q9U3U0) Evalue: 2e-13	83	2 (1)	27	37	4.2	2.52	2.52
contig03193:157:876:1 similar to 39S ribosomal protein L10, mitochondrial (Q9VPL3) Evalue: 1e-08	69	2	14.6	239			0.25
contig20152:1:159:2 similar to 60S ribosomal protein L37a (Q90YT0) Evalue: 1e-05	69	2	26.9	52	1.49		
contig20127:1:201:1 similar to 40S ribosomal protein S27a (P31753) Evalue: 3e-09	120	4 (2)	42.4	66	1.68		
contig19640:110:598:2 similar to 40S ribosomal protein S11 (P62280) Evalue: 3e-66	359	11 (7)	44.4	162	5.82	2.93	3.99
contig19506:94:324:1 similar to 40S ribosomal protein S17 (P49215) Evalue: 2e-32	160	8 (4)	41.6	77	6.88	3.65	5.09
contig19419:1:237:1 similar to 60S ribosomal protein L7a (Q2TBQ5) Evalue: 2e-25	170	6 (4)	41	78	9.99	6.36	3.94
contig17438:108:706:3 similar to 60S ribosomal protein L7a (Q4R5C2) Evalue: 1e-71	280	11 (7)	29.1	199	5.49	3.6	3.62
contig19279:1:281:1 similar to 60S acidic ribosomal protein P2 (O01725) Evalue: 6e-17	364	11 (5)	60.2	93	9.8		
contig00078:1:836:3 similar to 39S ribosomal protein L12, mitochondrial (Q7YR75) Evalue: 1e-26	106	2	9.4	277			0.23
contig18924:106:440:1 similar to 60S ribosomal protein L13 (Q962U1) Evalue: 1e-31	137	5 (3)	24.3	111	3	2.41	1.51
contig18137:1:342:1 similar to 60S ribosomal protein L13 (Q962U1) Evalue: 1e-14	208	6 (5)	24.8	113	7.76	2.85	3.06
contig18814:70:537:1 similar to 40S ribosomal protein S18 (Q8ISP0) Evalue: 4e-53	264	8 (6)	36.1	155	24.19	4.45	6.28
contig17018:79:477:1 similar to 40S ribosomal protein S18 (P35271) Evalue: 2e-39	148	3	21.2	132	0.8		0.48
contig18001:773:1333:1 similar to 39S ribosomal protein L18, mitochondrial (Q9H0U6) Evalue: 4e-23	130	3	19.9	186	0.56		
contig16544:1:963:1 similar to 60S ribosomal protein L5-1 (Q0JGY1) Evalue: 2e-83	68	2	4.7	320		0.19	0.41
contig14228:95:1012:2 similar to 60S ribosomal protein L5 (O76190) Evalue: 4e-81	794	24 (14)	48.9	305	4.7	8.32	4.46
contig17425:1:206:3 similar to 60S ribosomal protein L22 1 (Q54JE3) Evalue: 1e-05	333	9 (4)	44.8	67	6.99		1.49
contig07730:1:237:1 similar to 60S ribosomal protein L22-like 1 (Q5I0R6) Evalue: 3e-09	133	3	29.5	78	1.71		
contig04785:84:307:3 similar to 60S ribosomal protein L22-like 1 (Q5I0R6) Evalue: 2e-11	75	4 (1)	32.4	74	3.07		
contig16808:174:557:3 similar to 60S ribosomal protein L30 (P58374) Evalue: 7e-50	74	1	12.6	127	13.14	0.48	0.55
contig09849:196:521:1 similar to 40S ribosomal protein S15a (Q9AT34) Evalue: 2e-46	100	4 (3)	25.9	108	1.08		
contig09266:1:353:3 similar to 40S ribosomal protein S15Aa (P48149) Evalue: 2e-55	287	18 (6)	44	116	7.99		0.5
contig09093:1:762:3 similar to 40S ribosomal protein S4 (Q95V34) Evalue: 8e-123	857	47 (16)	58.9	253	9.72	17.01	19.92
contig06600:153:908:3 similar to 40S ribosomal protein S4, X isoform (Q642H9) Evalue: 2e-108	300	7	31	252	0.25	1.16	1.69
contig08742:102:554:3 similar to 40S ribosomal protein S15 (Q945U1) Evalue: 2e-49	54	1	12.7	150	3.6	0.4	0.71
contig07385:1:906:1 similar to 40S ribosomal protein SA (B5FXT6) Evalue: 5e-100	573	26 (10)	45.5	301	7.3	7.07	8.02
contig06667:1:709:2 similar to 28S ribosomal protein S34, mitochondrial (P82929) Evalue: 5e-08	62	2	8.5	235	0.26		0.12

contig05798:1:534:1 similar to 28S ribosomal protein S18b, mitochondrial (Q5TM62) Evalue: 8e-22	38	1	4.5	177	0.32	0.32		
contig05745:1:191:1 similar to 60S ribosomal protein L19 (Q3T0W9) Evalue: 9e-09	51	1	11.1	63	0.98	0.98		
contig03238:1:344:1 similar to 60S ribosomal protein L19 (Q3T0W9) Evalue: 2e-41	162	9 (5)	22.8	114	2			
contig05559:1:106:2 similar to 60S ribosomal protein L36 (Q9Y3U8) Evalue: 2e-07	60	2	45.7	35	3.43			
contig05395:1422:2312:3 similar to 39S ribosomal protein L43, mitochondrial (Q8N983) Evalue: 1e-26	199	4	16.9	296		0.45	0.45	
contig03688:1:470:3 similar to 39S ribosomal protein L27, mitochondrial (Q9P0M9) Evalue: 2e-11	118	3	21.9	155	1.11			
contig03602:1:363:2 similar to 60S ribosomal protein L27 (Q7ZV82) Evalue: 2e-43	150	5 (4)	33.3	120	2.21			
contig03311:1:336:2 similar to 60S ribosomal protein L13a (Q4R8Z2) Evalue: 1e-47	196	5	39.6	111	3.54	3.04	2.71	
contig01979:17:639:2 similar to 60S ribosomal protein L16 (Q9P720) Evalue: 2e-64	119	2	13	207	0.31		0.31	
contig03259:1:109:2 similar to 40S ribosomal protein S21 (Q6AZJ9) Evalue: 3e-06	106	3 (2)	31.4	35	6.88			
contig02633:61:263:1 similar to 60S ribosomal protein L38 (Q56FC8) Evalue: 5e-14	148	6 (3)	40.3	67	2.03			
contig02228:1:396:3 similar to 60S ribosomal protein L6-2 (Q9C9C6) Evalue: 4e-26	189	3	29	131	0.85		0.51	
contig00193:1:747:1 similar to 60S ribosomal protein L6 (Q6QMZ4) Evalue: 1e-47	476	16 (9)	35.1	248	4.99	5.08	4.58	
contig00034:1:710:3 similar to 60S ribosomal protein L8 (Q6RYS3) Evalue: 7e-90	384	11 (7)	43.4	235	5.24	4.7	4.81	
contig28047:1:322:2 similar to 40S ribosomal protein S20-2 (Q9STY6) Evalue: 3e-40	75	2 (1)	11.3	106	3.14	1.12	0.84	
contig28042:1:1314:1 similar to 60S ribosomal protein L3 (P49149) Evalue: 3e-167	200	4	13.3	437	5.35	1.48	1.14	
contig28174:1:216:1 similar to 60S ribosomal protein L24 (Q8ISQ3) Evalue: 1e-10	62	2	19.4	72	2.26	1.42	3.26	
contig27668:1:507:1 similar to 40S ribosomal protein S24 (Q962Q6) Evalue: 7e-30	119	3	21.4	168	1.8	1.17	0.92	
contig20885:1490:1867:2 similar to 39S ribosomal protein L53, mitochondrial (Q2HJF1) Evalue: 1e-08	74	2	16	125	0.77			
contig24239:1:906:1 similar to 39S ribosomal protein L39, mitochondrial (Q9VUJ0) Evalue: 5e-68	89	2	10	301	0.2			
contig17436:2625:3680:1 similar to 39S ribosomal protein L44, mitochondrial (Q9H9J2) Evalue: 4e-41	55	2	6.8	351			0.08	
contig04925:1:456:1 similar to 28S ribosomal protein S22, mitochondrial (Q9CXW2) Evalue: 4e-12	46	1	5.3	151			0.38	
contig01235:1:1357:2 similar to 60S ribosomal export protein NMD3 (Q99L48) Evalue: 3e-120	101	2	4.9	452		0.13	0.13	
<b>Lyases</b>								
contig28307:1:283:3 similar to N-acetylneuraminate lyase (Q5M905) Evalue: 9e-21	250	6 (3)	37.6	93	0.83	3.51	2.34	
contig25857:1:236:1 similar to N-acetylneuraminate lyase (Q6NYR8) Evalue: 5e-05	175	9 (3)	43.6	78	0.82	3.36	1.98	
contig24410:103:630:1 similar to Lactoylglutathione lyase (Q6P7Q4) Evalue: 1e-65	238	4	33.7	175	0.36	0.85	0.85	
contig24043:1:1018:1 similar to Adenylosuccinate lyase (P21265) Evalue: 8e-140	282	4	19.5	339	0.18	0.38	0.28	
contig22655:1:501:1 similar to Hydroxymethylglutaryl-CoA lyase, mitochondrial (Q8HXZ6) Evalue: 2e-57	123	2	15.7	166		0.42		
contig22495:127:1338:1 similar to Citrate lyase subunit beta-like protein, mitochondrial (Q8N0X4) Evalue: 7e-81	39	1	4	403			0.14	
contig21113:1:875:2 similar to Threo-3-hydroxyaspartate ammonia-lyase (P36007) Evalue: 1e-30	65	1	5.8	291			0.2	
contig19654:1:660:3 similar to Cystathionine gamma-lyase (P32929) Evalue: 6e-76	315	10 (5)	35.2	219	0.29	1.89	1.28	
contig18604:399:571:3 similar to Cystathionine gamma-lyase (Q60HG7) Evalue: 5e-12	161	3	57.1	56	3.2		1.6	
contig17470:1:446:3 similar to Adenylosuccinate lyase (P54822) Evalue: 1e-52	100	2	23.1	147	0.45			
contig10009:212:730:2 similar to Hydroxymethylglutaryl-CoA lyase, mitochondrial (Q8HXZ6) Evalue: 7e-49	232	4	24.9	173	0.38	0.9	0.9	
contig09291:1:184:2 similar to N-acetylneuraminate lyase (Q9BEC7) Evalue: 3e-08	129	4 (2)	41	61	2.49	2.49	1.3	
contig04869:1080:1491:3 similar to Cystathionine gamma-lyase (Q19QT7) Evalue: 1e-34	165	6 (2)	22.6	137	0.85	1.28	1.28	
contig04837:1:1460:3 similar to Histidine ammonia-lyase (P42357) Evalue: 0.0	435	9	27.2	485		0.72	0.44	
<b>Phosphatases</b>								
contig03773:1:1058:3 similar to Serine/threonine-protein phosphatase 4 catalytic subunit (P97470) Evalue: 7e-160	137	3 (2)	7.7	351	0.17			
contig00228:1:993:1 similar to Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform (P67776) Evalue: 3e-175	432	10 (8)	35.5	330	1.5	1.6	0.87	

## Supplementary

contig03101:248:1753:2 similar to Serine/threonine-protein phosphatase 2B catalytic subunit 3 (Q9VXF1) Evalue: 0.0	290	8 (6)	15.2	501	0.24	0.91	0.64
contig03265:1:795:1 similar to Serine/threonine-protein phosphatase 6 regulatory subunit 1 (Q9UPN7) Evalue: 4e-16	285	5	27.3	264	0.36	0.67	0.23
contig06657:137:1048:2 similar to Serine/threonine-protein phosphatase 2A regulatory subunit B- (Q28717) Evalue: 1e-97	76	2	13.2	303	0.2		0.18
contig17157:152:1162:2 similar to Serine/threonine-protein phosphatase PP1-beta (Q627N3) Evalue: 2e-174	264	6 (5)	20.5	336	1.69	0.59	1.86
contig20267:78:1724:3 similar to Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit epsilon isoform (Q16537) Evalue: 3e-169	654	13 (11)	29.4	548	0.76	0.9	1.05
contig27290:1:1093:1 similar to Serine/threonine-protein phosphatase 6 catalytic subunit (Q27884) Evalue: 6e-133	127	3	9.1	364	0.33		
contig26157:1:1731:1 similar to Serine/threonine-protein phosphatase 4 regulatory subunit 2-A (Q5M7D6) Evalue: 2e-18	391	11 (7)	17.7	576	0.85		
contig23488:1:638:3 similar to Serine/threonine-protein phosphatase PGAM5, mitochondrial (Q502L2) Evalue: 3e-70	103	3	18.5	211	0.3	0.48	0.3
contig23244:191:1180:2 similar to Serine/threonine-protein phosphatase PP1-beta catalytic subunit (Q6GQL2) Evalue: 6e-176	484	10 (9)	32.5	329	0.79	1.8	
contig24521:252:1445:3 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 8e-56	168	2	7.5	398			0.15
contig24336:1:650:3 similar to Lysophosphatidic acid phosphatase type 6 (Q9NPH0) Evalue: 1e-22	205	3	23.6	216	0.46	0.46	0.66
contig24098:128:1165:2 similar to Fructose-1,6-bisphosphatase isozyme 2 (Q9Z1N1) Evalue: 1e-124	649	17 (9)	39.7	345	1.4	2.42	2.73
contig23959:1:1527:1 similar to Prostatic acid phosphatase (Q8CE08) Evalue: 7e-25	87	2	6.5	508		0.12	0.12
contig23850:103:1917:1 similar to Tyrosine-protein phosphatase non-receptor type 9 (P43378) Evalue: 1e-124	99	2	4.1	604	0.1		
contig23771:287:1256:2 similar to Protein phosphatase 1B (O62830) Evalue: 1e-110	267	7 (6)	22.6	323	1.93	0.74	0.99
contig21118:1:529:2 similar to 14 kDa phosphohistidine phosphatase (P83468) Evalue: 1e-35	159	3	19.4	175	0.58		
contig20976:1:507:1 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 4e-06	267	8 (4)	25.6	168	0.34	4.29	4.12
contig20498:1:1715:3 similar to Probable serine/threonine-protein phosphatase PP2A regulatory subunit (Q09543) Evalue: 0.0	1027	24 (18)	39.8	570	1.66	2.21	1.71
contig18980:1:815:2 similar to Inositol monophosphatase (O77591) Evalue: 6e-69	133	3	11.8	271	0.54	0.49	0.35
contig18927:1:1035:2 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 2e-48	629	32 (10)	46.2	344		6.53	5.21
contig18815:36:1291:3 similar to Tyrosine-protein phosphatase non-receptor type 4 (Q9WU22) Evalue: 4e-41	880	27 (14)	46.7	418	0.46	2.95	3.31
contig18740:1:458:1 similar to Inorganic pyrophosphatase (O77460) Evalue: 1e-31	135	5 (3)	23.7	152		1.2	1.01
contig18009:1:1983:1 similar to Probable protein phosphatase CG10417 (Q7K4Q5) Evalue: 1e-61	120	4 (3)	6.5	660	0.14		
contig17628:1:923:3 similar to Inositol monophosphatase 3 (Q80V26) Evalue: 1e-52	141	3	10.5	306	0.33		
contig12425:1:708:1 similar to Bis(5--nucleosyl)-tetrakisphosphate [asymmetrical] (Q6PEC0) Evalue: 8e-15	448	12 (9)	33.6	235		2.11	3.33
contig09593:1:2236:2 similar to Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (P97675) Evalue: 1e-122	98	2	3.1	744		0.08	
contig09436:109:1905:1 similar to Phosphatidylinositol phosphatase SAC1 (A6QL88) Evalue: 4e-144	54	1	1.8	598		0.1	
contig06214:126:2222:3 similar to Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (P15396) Evalue: 4e-96	54	2	3.6	698			0.08
contig06192:1:1250:3 similar to Putative inositol monophosphatase 3 (Q9VYF2) Evalue: 6e-48	640	13 (11)	33.3	415	0.63	1.16	0.88
contig05914:107:904:2 similar to Protein phosphatase 1 regulatory subunit 7 (Q3T0W4) Evalue: 2e-69	338	8	36.5	266	1.29		
contig05220:1:642:1 similar to Lysosomal acid phosphatase (B1H1P9) Evalue: 3e-07	117	3 (2)	11.3	213		0.31	0.31
contig04652:1:1122:1 similar to Tartrate-resistant acid phosphatase type 5 (P13686) Evalue: 2e-72	102	2	6.4	373	0.24		
contig04436:1:274:1 similar to Lysophosphatidic acid phosphatase type 6 (Q9NPH0) Evalue: 8e-06	90	2	27.5	91			0.74
contig04025:1:1355:3 similar to Type II inositol-1,4,5-trisphosphate 5-phosphatase (P32019) Evalue: 1e-120	59	2	2.7	450			0.06
contig03966:247:390:1 similar to Protein phosphatase 1 regulatory subunit 7 (Q6DIQ3) Evalue: 2e-07	109	2	63.8	47	1.83		1.83
contig00318:391:1335:1 similar to Protein tyrosine phosphatase type IVA 1 (Q5R7J8) Evalue: 4e-52	59	2	10.2	314	0.2		
contig02870:760:1365:2 similar to Protein-tyrosine phosphatase mitochondrial 1 (Q8WUK0) Evalue: 4e-32	79	2	10	201	0.31		
contig02696:1:1045:2 similar to Protein phosphatase methyltransferase 1 (Q8BVQ5) Evalue: 8e-76	110	2	7.8	347	0.18	0.18	

contig02624:1:558:1 similar to Inorganic pyrophosphatase (Q9D819) Evalue: 1e-52	395	9 (5)	43.2	185	1.63	1.73	1.94
contig02499:128:721:2 similar to Prostatic acid phosphatase (P15309) Evalue: 6e-25	503	15 (7)	57.6	198	1.61	6.37	11
contig01713:1:894:1 similar to Prostatic acid phosphatase (A6H730) Evalue: 2e-28	251	6 (4)	14.8	297	0.32	1.73	1.71
contig03564:118:1004:1 similar to Prostatic acid phosphatase (A6H730) Evalue: 1e-17	501	13 (9)	47.8	295	0.21	1.6	2.23
<b>Protein biosynthesis/translational elongation</b>							
contig26452:1:179:2 similar to Elongation factor 1-beta~ (P29522) Evalue: 2e-21	315	9 (5)	84.7	59	14.85	27.26	17.24
contig26119:1:477:1 similar to Elongation factor 1-alpha (Q6L202) Evalue: 2e-35	65	2	14.6	158		0.6	
contig25681:172:795:1 similar to Elongation factor Ts, mitochondrial (Q9CZR8) Evalue: 4e-38	293	5	30.3	208	1.22	0.94	0.49
contig23992:1:1184:3 similar to Elongation factor 1-gamma (P29694) Evalue: 1e-68	107	3	7.1	394		0.15	0.24
contig22711:32:1325:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	1242	109 (22)	65.7	431	32.72	38.85	44.47
contig22674:1:121:3 similar to Elongation factor 1-beta (P12262) Evalue: 1e-05	69	2	20.5	39	1.68	3.21	1.68
contig21455:1:1099:2 similar to Elongation factor 2 (O14460) Evalue: 6e-148	135	5 (4)	9.3	366		0.53	0.71
contig20348:117:860:3 similar to Elongation factor 1-gamma (Q90YC0) Evalue: 6e-39	701	22 (10)	65.3	248	10.64	5.51	4.35
contig18162:1:459:2 similar to Elongation factor 1-gamma (Q4R7H5) Evalue: 1e-52	580	15 (8)	58.6	152	4.59	7.19	3.56
contig15102:1:321:1 similar to Elongation factor 1-alpha (P02993) Evalue: 3e-24	65	2	15.1	106	0.67	0.96	0.58
contig14931:1:749:3 similar to Elongation factor 2 (P29691) Evalue: 9e-69	67	4 (2)	6	248	0.61	0.97	1.22
contig11548:1:1628:3 similar to Elongation factor 2 (P09445) Evalue: 0.0	1594	58 (28)	64.8	542	10.13	14	11.31
contig11452:1:1044:1 similar to Elongation factor 2 (Q96X45) Evalue: 4e-124	81	2	8.1	347		0.27	0.18
contig06913:1:1377:1 similar to Elongation factor Tu, mitochondrial (Q8BFR5) Evalue: 2e-111	84	2	5	458	0.28	0.13	0.28
contig04720:115:1504:1 similar to Elongation factor Tu, mitochondrial (P49411) Evalue: 8e-148	683	15 (12)	33.3	463	2.71	1.84	1.66
contig03955:1:1081:2 similar to Elongation factor 2 (P09445) Evalue: 0.0	1330	50 (21)	71.3	359	13.16	20.1	18.1
contig03510:1:861:1 similar to Elongation factor 1-alpha 1 (P08736) Evalue: 4e-38	173	4	14	286		0.73	
contig03464:1:333:1 similar to Elongation factor 2 (Q874B9) Evalue: 3e-40	95	3 (2)	22.5	111		1.1	0.64
contig01731:1:672:1 similar to Elongation factor 1-beta (P30151) Evalue: 7e-41	200	4	28.3	223	0.29	1.14	1.14
contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	372	23 (8)	20.8	457	1.55	0.98	1.07
<b>Kinases</b>							
<b>protein kinase superfamily. CAMK Ser/Thr protein kinase family.</b>							
contig24868:62:1564:2 similar to Titin (Q9I7U4) Evalue: 4e-90	232	4	11.4	500		0.31	0.31
contig19358:1:1646:3 similar to Titin (Q9I7U4) Evalue: 1e-95	945	21 (19)	50.1	547		5.46	4.1
contig12603:1:673:1 similar to Titin (A2ASS6) Evalue: 1e-28	302	7 (5)	40.2	224		2.94	3.33
contig07735:1:549:1 similar to Titin (A2ASS6) Evalue: 3e-25	137	3	25.1	183		1.28	0.92
contig05265:1:769:2 similar to Titin (Q9I7U4) Evalue: 9e-53	42	1	5.1	256			0.36
contig00931:1:255:3 similar to Titin (Q9I7U4) Evalue: 4e-17	35	1	11.9	84		0.74	
contig23009:438:1457:3 similar to Myosin light chain kinase, smooth muscle (P29294) Evalue: 1e-99	135	4 (3)	8.2	340		0.28	0.28
contig19615:1:999:3 similar to Myosin light chain kinase, smooth muscle (Q6PDN3) Evalue: 3e-29	263	4	18.7	332		2.41	2.91
contig04782:1:900:2 similar to Myosin light chain kinase, smooth muscle (Q6PDN3) Evalue: 6e-16	309	7 (6)	23.4	299		1.16	0.47
contig02065:1:495:1 No distinct annotation Best-hit: Myosin light chain kinase, smooth muscle (P11799) Evalue: 9e-11	91	2	13.4	164	0.82	0.6	
<b>choline/ethanolamine kinase family.</b>							
contig23674:1:859:2 similar to Ethanolamine kinase 1 (Q9D4V0) Evalue: 3e-30	144	3	10.9	285		0.32	0.21
contig17094:1:1106:3 similar to Ethanolamine kinase 1 (Q9HBU6) Evalue: 4e-32	62	1	4.4	367		0.16	0.41
contig24781:167:1056:2 similar to Ethanolamine kinase 1 (Q9D4V0) Evalue: 4e-28	103	2	10.5	296	0.58	0.2	0.2



contig24002:1:1137:1 similar to Ethanolamine kinase 1 (Q9HBU6) Evalue: 7e-35	231	6	15.1	378	0.69	0.39
contig24570:1:334:2 similar to Ethanolamine kinase 1 (Q9HBU6) Evalue: 3e-11	53	1	13.5	111	0.54	0.89
contig22839:1:597:1 similar to Ethanolamine kinase (P54352) Evalue: 1e-26	133	3	15.7	198	0.96	1.07
contig21105:149:1246:2 similar to Ethanolamine kinase 1 (Q9HBU6) Evalue: 1e-60	31	1	2.7	365	0.16	
<b>others</b>						
contig03484:1:1827:2 similar to 5--AMP-activated protein kinase catalytic subunit alpha-1 (P54645) Evalue: 8e-172	166	3	7.4	608	0.26	0.15 0.21
contig07985:2341:3165:3 similar to 5--AMP-activated protein kinase subunit beta-2 (O43741) Evalue: 3e-69	210	4 (3)	16.4	274	0.51	0.51 0.51
contig23728:238:1767:1 similar to 5--AMP-activated protein kinase subunit gamma-2 (Q91WG5) Evalue: 7e-106	454	9	23.2	509	0.43	0.9 0.37
contig06677:1:863:1 similar to cAMP-dependent protein kinase type II regulatory subunit (Q26619) Evalue: 3e-54	170	4	14.3	287		0.58 0.73
contig23484:1:282:1 similar to cAMP-dependent protein kinase type II regulatory subunit (P81900) Evalue: 2e-19	156	3	36.6	93	1.32	0.75
contig07391:1:881:3 similar to cAMP-dependent protein kinase catalytic subunit alpha (P05132) Evalue: 1e-141	83	3	9.9	293		0.32
contig05197:428:2692:2 similar to Ribosomal protein S6 kinase alpha-2 (Q15349) Evalue: 0.0	66	2	4.2	754		0.08
contig19003:1:1127:3 similar to [Pyruvate dehydrogenase [lipoamide]] kinase, mitochondrial (P91622) Evalue: 1e-107	57	1	4	374		0.16
contig27476:119:1849:2 similar to Glycerol kinase (Q63060) Evalue: 6e-152	564	14 (12)	29.5	576	0.57	0.97 1.23
contig26856:74:535:2 similar to Nucleoside diphosphate kinase B (Q5RFH3) Evalue: 2e-64	203	4	31.4	153	16.55	2.54 1.34
contig24274:1:500:3 similar to Nucleoside diphosphate kinase (O57535) Evalue: 7e-61	143	4 (3)	23	165	0.66	
contig26753:57:848:3 similar to Adenylate kinase 2, mitochondrial (Q7QJX9) Evalue: 2e-77	235	5	23.2	263	0.48	0.7 0.53
contig26667:190:1299:1 similar to Adenosine kinase (P55263) Evalue: 3e-82	293	5	19	369	0.34	0.83 0.55
contig25988:1:904:3 similar to Mitogen-activated protein kinase 1 (P46196) Evalue: 9e-134	253	7 (6)	17	300	0.31	1.08 0.57
contig17990:1:716:3 similar to Phosphoenolpyruvate carboxykinase, cytosolic [GTP] (P07379) Evalue: 9e-107	608	15 (11)	62.2	238	0.27	3.75 2.32
contig25321:1:771:1 similar to Phosphoenolpyruvate carboxykinase [GTP], mitochondrial (Q8BH04) Evalue: 4e-94	365	13 (8)	37.5	256		1.59 1.09
contig24956:1:847:3 similar to Phosphoglycerate kinase (P51903) Evalue: 2e-108	978	26 (15)	66.9	281	1.77	7.13 9.43
contig24273:237:1724:3 similar to Uncharacterized aarF domain-containing protein kinase 5 (Q80V03) Evalue: 3e-127	402	9 (8)	22.6	495	0.24	0.65 0.47
contig24259:1:1301:3 similar to Hexokinase type 2 (Q9NFT7) Evalue: 9e-113	700	13 (12)	36.8	432	0.49	1.7 0.85
contig23002:90:1244:3 similar to N-acetyl-D-glucosamine kinase (Q3SZM9) Evalue: 3e-73	131	2	11.2	384		0.16
contig22329:562:898:1 similar to Phosphoenolpyruvate carboxykinase [GTP], mitochondrial (Q8BH04) Evalue: 4e-30	145	3	26.8	112		1.03 0.6
contig21179:817:1959:1 similar to Casein kinase II subunit alpha (P28020) Evalue: 2e-169	329	7 (6)	22.1	380	0.54	0.54 0.54
contig06479:200:1441:2 similar to Calcium/calmodulin-dependent protein kinase type II alpha chain (Q00168) Evalue: 6e-148	63	2	5.1	414		0.14
contig21172:45:662:3 similar to Calcium/calmodulin-dependent protein kinase type II delta chain (Q95266) Evalue: 6e-44	163	3	29.3	205		0.5 0.5
contig20692:99:919:3 similar to Mitogen-activated protein kinase 14 (P47812) Evalue: 2e-71	86	3 (2)	6.6	273		0.35
contig20576:1:610:2 similar to Mitogen-activated protein kinase 14A (O62618) Evalue: 2e-54	86	2	9.9	202	0.31	
contig20126:1:839:3 similar to Dual specificity mitogen-activated protein kinase kinase 1 (Q91447) Evalue: 9e-94	85	2	6.8	278		0.32 0.22
contig20448:1:1208:3 similar to Protein kinase C and casein kinase substrate in neurons protein 2 (Q9WVE8) Evalue: 3e-60	57	2	5.2	402	0.15	
contig07704:1:698:2 similar to Arginine kinase (O96507) Evalue: 5e-41	110	2	9.5	232		0.27
contig10556:1:458:3 similar to Arginine kinase (P91798) Evalue: 2e-52	169	7 (3)	25.8	151		1.01 2.09
contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	1592	85 (26)	73.3	363	1.76	37.1 35.73
contig19697:159:767:3 similar to cGMP-dependent protein kinase 1, beta isozyme (P21136) Evalue: 2e-42	127	2	14.3	203		0.3
contig19523:1:637:3 similar to Traf2 and NCK-interacting protein kinase (P83510) Evalue: 3e-102	31	1	3.3	211	0.42	
contig19251:1138:1617:1 similar to UMP-CMP kinase (Q29561) Evalue: 3e-40	276	10 (6)	45.3	159	0.7	3.47 3.09
contig18837:256:1929:1 similar to STE20/SPS1-related proline-alanine-rich protein kinase (O88506) Evalue: 7e-152	90	2	4.1	557	0.11	0.11
contig18818:1:355:2 similar to Phosphoglycerate kinase (P38667) Evalue: 2e-31	185	7 (5)	39.8	118		2.94 2.94

contig18751:1:549:1 similar to Casein kinase II subunit beta (Q91398) Evalue: 2e-76	45	1	4.9	182	0.35	0.32		
contig18632:105:1412:3 similar to MAP kinase-activated protein kinase 3 (Q16644) Evalue: 7e-136	150	3	7.6	435		0.21	0.14	
contig17945:1:686:1 similar to Cell division cycle 2-like protein kinase 6 (Q8BWD8) Evalue: 7e-105	37	1	3.9	228			0.26	
contig17939:1:834:1 similar to Pyruvate kinase isozymes M1/M2 (P14618) Evalue: 7e-94	520	14 (10)	54.5	277	0.89	3.7	3.09	
contig05711:251:901:2 similar to Pyruvate kinase isozyme M1 (P11979) Evalue: 7e-73	736	19 (11)	70	217	2.56	6.69	8.92	
contig15875:1:392:3 similar to Pyruvate kinase muscle isozyme (Q92122) Evalue: 6e-34	140	3 (2)	18.5	130		1.73	1.5	
contig17553:79:458:1 similar to Probable adenylate kinase isoenzyme F38B2.4 (Q20140) Evalue: 2e-21	353	8 (6)	61.9	126	0.57	4.4	3.1	
contig17526:1:221:3 similar to Striated muscle-specific serine/threonine-protein kinase (Q62407) Evalue: 2e-04	150	4 (3)	51.4	72		8.27	3.06	
contig12871:1:533:3 similar to Probable serine/threonine-protein kinase fhkD (Q54MH0) Evalue: 2e-16	28	1	4	177		0.51	0.85	
contig11796:151:1318:1 similar to RAC serine/threonine-protein kinase (Q8INB9) Evalue: 1e-123	357	8	19.3	389	0.63	0.75	0.63	
contig11071:1:467:3 similar to RAC serine/threonine-protein kinase (Q8INB9) Evalue: 5e-52	137	3	30.5	154			0.69	
contig05493:1:977:3 similar to Serine/threonine-protein kinase 6 (Q91820) Evalue: 2e-94	88	2	4.9	324	0.18		0.18	
contig10954:1:1724:3 similar to Serine/threonine-protein kinase PLK1 (Q07832) Evalue: 1e-134	233	5	13.4	573	0.27			
contig10151:307:795:1 similar to Phosphatidylinositol-5-phosphate 4-kinase type-2 alpha (O70172) Evalue: 6e-44	62	2	19	163			0.39	
contig09415:132:1109:3 similar to Guanylate kinase (Q64520) Evalue: 9e-48	302	5	22.5	325	0.5	0.54	0.41	
contig09098:1:1366:2 similar to Pantothenate kinase 1 (Q8TE04) Evalue: 6e-117	71	2	3.5	454		0.13		
contig09005:196:1206:1 similar to Ribose-phosphate pyrophosphokinase 2 (Q5XGI0) Evalue: 1e-158	181	5 (4)	15.2	336	0.96	0.53	0.28	
contig07429:1:196:3 similar to Adenylate kinase isoenzyme 4, mitochondrial (Q9WUS0) Evalue: 3e-07	71	1	21.9	64		1.02	2.94	
contig06346:1:971:3 similar to Pyridoxal kinase (O46560) Evalue: 3e-83	196	4	15.8	322	0.3	0.42	0.69	
contig06074:131:301:2 similar to Casein kinase II subunit beta (P67874) Evalue: 3e-28	166	3 (2)	52.6	57	2.49			
contig05837:177:528:3 similar to Dual specificity mitogen-activated protein kinase kinase 2 (P36506) Evalue: 7e-17	62	2	17.1	117	0.6	0.6		
contig05524:314:1363:2 similar to Dual specificity mitogen-activated protein kinase kinase 6 (P52564) Evalue: 2e-88	56	2	4.9	349			0.17	
contig05722:1:254:3 similar to Galactokinase (P51570) Evalue: 8e-15	156	3 (2)	32.5	83		0.9		
contig04160:1:524:3 similar to Nucleoside diphosphate kinase (Q7Z8P9) Evalue: 8e-47	119	3	22.5	173	0.6			
contig04032:108:1064:3 similar to Galactokinase (P51570) Evalue: 2e-82	230	5	27.9	319		0.74	0.57	
contig02305:40:642:1 similar to Phosphomevalonate kinase (Q2KIU2) Evalue: 2e-47	146	3	18.5	200		0.64	0.94	
contig01873:1:1761:1 similar to Integrin-linked protein kinase (Q5R5V4) Evalue: 9e-164	459	13 (11)	21.8	586		0.69	0.69	
contig01094:1:1119:1 similar to Phosphatidylinositol 4-kinase type 2-beta (Q28G26) Evalue: 2e-112	94	2	6.7	373	0.16	0.16	0.34	
contig00645:1:1135:2 similar to Glycogen synthase kinase-3 beta (P49841) Evalue: 2e-161	119	2	8.2	377	0.71	0.24	0.5	
contig00575:1:1603:3 similar to 6-phosphofructokinase (P52034) Evalue: 9e-177	230	5	11.3	533		0.31	0.18	
<b>Synthetases</b>								
contig22669:1:853:2 similar to Phenylalanyl-tRNA synthetase alpha chain (Q1JPX3) Evalue: 2e-142	123	3	15.9	283		0.34	0.34	
contig25049:1:1434:3 similar to Methionyl-tRNA synthetase, cytoplasmic (Q6PF21) Evalue: 0.0	339	7 (6)	17.2	477		0.5	0.19	
contig27761:1:1391:2 similar to Acyl-CoA synthetase family member 2, mitochondrial (Q0P4F7) Evalue: 3e-105	132	4 (3)	9.3	463		0.2	0.28	
contig28195:1:158:1 similar to Farnesyl pyrophosphate synthetase (Q8WMY2) Evalue: 4e-06	84	2	46.2	52		1.49	1.49	
contig22391:1:1506:1 similar to Acyl-CoA synthetase short-chain family member 3, mitochondrial (Q14DH7) Evalue: 2e-146	758	17 (11)	34.7	501		1.21	0.76	
contig22391:1521:1949:3 similar to Acyl-CoA synthetase short-chain family member 3, mitochondrial (Q9H6R3) Evalue: 1e-26	132	4 (3)	25.4	142		1.2	1.2	
contig28138:1:639:2 similar to Acyl-CoA synthetase family member 2, mitochondrial (Q17QJ1) Evalue: 2e-44	164	4 (3)	23.6	212		0.48	0.3	
contig25040:1:552:1 similar to Leucyl-tRNA synthetase, cytoplasmic (Q8BMJ2) Evalue: 5e-45	105	1	8.7	184	0.16	0.32	0.51	
contig18328:1:1505:3 similar to Leucyl-tRNA synthetase, cytoplasmic (Q8BMJ2) Evalue: 9e-156	241	4	12.2	500		0.31		
contig22421:72:1191:3 similar to Bifunctional aminoacyl-tRNA synthetase (Q8CGC7) Evalue: 7e-161	101	3	7.5	373	0.25		1.02	

## Supplementary

contig24976:1:1655:3 similar to Bifunctional aminoacyl-tRNA synthetase (P07814) Evalue: 0.0	73	2	7.1	550	0.5	0.26	0.62
contig18749:1:332:3 similar to Bifunctional aminoacyl-tRNA synthetase (Q8CGC7) Evalue: 1e-24	33	1	15.5	110	0.54	0.81	0.54
contig18041:1:821:1 similar to Bifunctional 3--phosphoadenosine 5--phosphosulfate synthetase 2 (O88428) Evalue: 1e-101	367	6	32.6	273	1.5	1.5	1.02
contig25595:1:302:3 similar to Lysyl-tRNA synthetase (Q15046) Evalue: 2e-26	286	9 (7)	71	100	1.49	7.36	1.19
contig21404:104:1870:2 similar to Probable phenylalanyl-tRNA synthetase beta chain (Q9VCA5) Evalue: 0.0	322	7	14.3	588	0.47	0.61	0.43
contig19235:1:1086:2 similar to Histidyl-tRNA synthetase, cytoplasmic (Q61035) Evalue: 7e-113	156	4	13	361	0.42	0.6	0.34
contig27815:1:276:1 similar to GDP-L-fucose synthetase (Q5RBE5) Evalue: 2e-26	152	3	42.9	91	0.77	1.36	0.77
contig22889:238:502:1 similar to Glutamine synthetase (P15103) Evalue: 1e-27	107	1	18.2	88	0.7		0.7
contig19764:122:1843:2 similar to Glutaminyl-tRNA synthetase (Q3MHH4) Evalue: 0.0	535	11	21.6	573	0.88	0.7	0.55
contig17807:1:460:2 similar to Glutamine synthetase (P51121) Evalue: 9e-67	352	12 (6)	44.1	152	3.13	6.42	6.33
contig19028:34:2268:1 similar to Glycyl-tRNA synthetase (Q9CZD3) Evalue: 0.0	988	24 (20)	35.3	744	1.67	2.5	2.05
contig25577:1:379:3 similar to Glutamine synthetase (Q04831) Evalue: 4e-55	82	3	20.8	125	0.89	1.37	1.42
contig14306:1:1517:3 similar to Isoleucyl-tRNA synthetase, cytoplasmic (Q8BU30) Evalue: 1e-147	497	12	27.6	504	0.18	1.36	0.38
contig20314:1:1571:3 similar to Tyrosyl-tRNA synthetase, cytoplasmic (Q5ZJ08) Evalue: 5e-138	471	10 (9)	22.4	522	0.72	0.78	0.82
contig23679:1:1328:3 similar to Threonyl-tRNA synthetase, cytoplasmic (P26639) Evalue: 0.0	737	17 (14)	39.5	441	0.45	1.89	1.59
contig27426:1:751:1 similar to Threonyl-tRNA synthetase, cytoplasmic (P52709) Evalue: 5e-96	239	7 (6)	30	250	0.25	0.87	0.37
contig25477:1:359:3 similar to Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 (P31230) Evalue: 1e-40	163	2	26.3	118	1.22	1.22	1.22
contig23983:208:1734:1 similar to Seryl-tRNA synthetase, cytoplasmic (Q6P799) Evalue: 0.0	834	14	36	508	1.05	1.55	1.87
contig23963:1:1373:3 similar to Adenylosuccinate synthetase isozyme 1 (A5PJR4) Evalue: 6e-156	481	17 (9)	25.4	456	0.46	1.56	1.93
contig20645:1:1011:1 similar to Phosphoribosyl pyrophosphate synthetase-associated protein 2 (O60256) Evalue: 2e-115	125	3	12.8	336	0.29	0.29	
contig27048:107:1019:2 similar to Tryptophanyl-tRNA synthetase, cytoplasmic (Q09692) Evalue: 2e-108	67	2	5.9	304	0.31	0.2	0.29
contig25915:1:1287:1 similar to Alanyl-tRNA synthetase, mitochondrial (P36428) Evalue: 8e-96	98	2	5.6	428			0.14
contig25444:1:752:2 similar to Acyl-CoA synthetase family member 2, mitochondrial (Q0P4F7) Evalue: 2e-60	130	4 (3)	13.2	250			0.41
contig10967:1:1363:2 similar to Acyl-CoA synthetase family member 2, mitochondrial (Q0P4F7) Evalue: 1e-97	437	8	28.5	453		0.65	0.55
contig24827:1:1009:1 similar to Glutamine-dependent NAD(+) synthetase (Q5ZMA6) Evalue: 4e-107	46	1	3.3	336			0.18
contig23979:1:632:3 similar to Methionyl-tRNA synthetase, cytoplasmic (P56192) Evalue: 3e-30	109	3	14.8	209			0.48
contig21078:63:1952:3 similar to Glycyl-tRNA synthetase 1 (P38088) Evalue: 0.0	62	2	4.5	629			0.09
contig20071:1:724:2 similar to Geranylgeranyl pyrophosphate synthetase (Q6F596) Evalue: 7e-83	58	2	7.9	240	0.26		
contig17934:116:751:2 similar to Phenylalanyl-tRNA synthetase alpha chain B (Q7SYV0) Evalue: 1e-37	111	2	12.7	212			0.3
contig12099:1:943:1 similar to Probable alanyl-tRNA synthetase, cytoplasmic (O13914) Evalue: 4e-129	70	2	5.1	314			0.19
contig10119:26:824:2 similar to Leucyl-tRNA synthetase, cytoplasmic (Q9P2J5) Evalue: 2e-79	42	1	7.5	266	0.22	0.22	
contig09790:1:361:1 similar to Probable glutamine-dependent NAD(+) synthetase (Q9VYA0) Evalue: 8e-40	73	1	16.7	120		0.75	0.5
contig09260:357:1538:3 similar to Squalene synthetase (Q32KR6) Evalue: 1e-52	342	8	23.4	393		0.8	1
contig08733:1:259:2 similar to Lysyl-tRNA synthetase (Q99MN1) Evalue: 2e-26	84	2	44.7	85	1.57	0.87	3.81
contig06355:1:698:3 similar to Lysyl-tRNA synthetase (P37879) Evalue: 3e-105	199	8 (6)	26.3	232	0.26	0.79	0.59
contig08003:128:594:2 similar to Valyl-tRNA synthetase (P49696) Evalue: 1e-64	116	3	22.6	155		0.7	
contig07143:1:312:1 similar to Probable GDP-L-fucose synthetase (Q9W1X8) Evalue: 4e-17	48	2 (1)	12.5	104		0.58	0.58
contig06554:181:1740:1 similar to Asparaginylyl-tRNA synthetase, cytoplasmic (Q4R4Z1) Evalue: 0.0	690	15 (10)	27.7	519	1.25	1.11	0.85
contig06400:1:310:3 similar to Farnesyl pyrophosphate synthetase (P08836) Evalue: 9e-09	95	2	20.6	102		0.65	
contig06173:1:239:3 similar to Tryptophanyl-tRNA synthetase, cytoplasmic (Q12109) Evalue: 7e-19	151	2	41.8	79	0.9		
contig05090:194:2243:2 similar to Acetyl-coenzyme A synthetase (A0KNI2) Evalue: 0.0	220	6	13.9	683		0.28	0.18

contig04654:3427:4479:1 similar to Asparagine synthetase [glutamine-hydrolyzing] (Q5ZJU3) Evalue: 2e-97	149	4	12.6	350	0.38	0.46	0.49
contig04654:2793:3308:2 similar to Asparagine synthetase [glutamine-hydrolyzing] (Q5ZJU3) Evalue: 2e-59	127	3	19.9	171		0.58	0.58
contig04545:79:1407:1 similar to Seryl-tRNA synthetase, cytoplasmic (O14018) Evalue: 3e-154	105	2	6.3	442		0.13	0.21
contig04349:1:280:1 similar to Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 (O54873) Evalue: 1e-07	78	1	11.8	93	0.7	0.7	0.7
contig03789:1:274:2 similar to Histidyl-tRNA synthetase, cytoplasmic (P70076) Evalue: 2e-29	80	1	14.3	91	0.8	0.68	0.68
contig02887:1:674:3 similar to Tryptophanyl-tRNA synthetase, cytoplasmic (P32921) Evalue: 2e-63	69	2	9.4	224	0.28	0.28	
contig02872:1:298:2 similar to Lysyl-tRNA synthetase (Q99MN1) Evalue: 1e-33	194	5 (4)	62.6	99	1.22	2.77	1.22
contig02702:1:1654:2 similar to Valyl-tRNA synthetase (Q04462) Evalue: 9e-157	552	10 (9)	20.5	550		0.87	0.34
contig02556:95:880:2 similar to Proline synthetase co-transcribed bacterial homolog protein (Q3T0G5) Evalue: 5e-67	55	1	5	261		0.22	
contig02428:204:2093:3 similar to Arginyl-tRNA synthetase, cytoplasmic (P54136) Evalue: 0.0	570	14 (12)	21.5	629	0.45	0.92	0.39
contig02179:369:2489:3 similar to Alanyl-tRNA synthetase, cytoplasmic (Q8BGQ7) Evalue: 0.0	889	18	29.6	706	0.46	1.59	0.77
contig02152:1:1564:2 similar to Acyl-CoA synthetase family member 2, mitochondrial (Q0P4F7) Evalue: 1e-125	279	7	17.3	520		0.46	0.39
contig01428:1:1633:2 similar to Cysteinyl-tRNA synthetase, cytoplasmic (Q9ER72) Evalue: 3e-164	423	8 (7)	19.1	544	0.17	0.51	0.36
contig01301:1:967:2 similar to Bifunctional 3--phosphoadenosine 5--phosphosulfate synthetase (Q27128) Evalue: 5e-140	403	9 (8)	31.5	321	1.01	1.29	0.39
contig00853:1:651:1 similar to Tryptophanyl-tRNA synthetase, cytoplasmic (P17248) Evalue: 2e-87	150	3	21.8	216	0.46	0.46	0.46
contig00802:105:1658:3 similar to Aspartyl-tRNA synthetase, cytoplasmic (P15178) Evalue: 0.0	881	20 (15)	32.5	517	1.46	2.36	2.86
contig00650:1:1097:3 similar to S-adenosylmethionine synthetase isoform type-2 (Q4R924) Evalue: 2e-149	450	13 (9)	35.2	364	1.18	1.44	1.18
<b>Hydrolases</b>							
contig23949:1:249:1 similar to Deoxyuridine 5--triphosphate nucleotidohydrolase (P70583) Evalue: 4e-29	238	6 (4)	59.8	82	3.29		
contig04368:251:997:2 similar to Platelet-activating factor acetylhydrolase IB subunit beta (Q5ZMS2) Evalue: 2e-30	147	4 (3)	15.3	248			0.41
contig03621:1:428:3 similar to Abhydrolase domain-containing protein 11 (Q0V9K2) Evalue: 7e-32	117	2	18.4	141			0.47
contig24384:282:1636:3 similar to Leukotriene A-4 hydrolase (Q3SZH7) Evalue: 6e-89	114	2	6.9	451			0.13
contig25953:1:210:1 similar to N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (O94760) Evalue: 1e-09	27	1	8.6	70		0.42	
contig28107:1:1008:2 similar to Epoxide hydrolase 4 (Q8IUS5) Evalue: 6e-71	195	6 (5)	15.2	335	0.63		0.92
contig18100:59:1273:2 similar to 3-hydroxyisobutyryl-CoA hydrolase, mitochondrial (Q58EB4) Evalue: 3e-110	124	3	8.9	404		0.38	0.23
contig16668:1:921:1 similar to Epoxide hydrolase 4 (Q8IUS5) Evalue: 4e-68	373	9 (7)	31.7	306		1.88	1.47
contig06216:1:1086:1 similar to Serine hydrolase-like protein (A2BGU9) Evalue: 2e-51	114	3	8	361		0.26	0.47
contig27667:1:1009:2 similar to Epoxide hydrolase 4 (Q8IUS5) Evalue: 6e-70	254	7 (6)	20.6	335		0.78	0.93
contig25610:43:1629:1 similar to Fatty-acid amide hydrolase 2 (Q6GMR7) Evalue: 3e-119	86	2	4	528		0.11	0.17
contig12440:1:1012:3 similar to Leukotriene A-4 hydrolase (Q3SZH7) Evalue: 3e-80	45	1	2.1	336		0.16	
contig25278:1:565:2 similar to Leukotriene A-4 hydrolase (P09960) Evalue: 2e-30	120	2	17.1	187		0.51	0.51
contig22544:1:1273:2 similar to Ectonucleoside triphosphate diphosphohydrolase 2 (O55026) Evalue: 7e-39	377	8	19.9	423		1.11	0.69
contig25107:1:932:3 similar to Fumarylacetoacetate hydrolase domain-containing protein 1 (Q8R0F8) Evalue: 8e-64	132	2	9.7	309		0.2	0.32
contig24441:1:655:2 similar to Hydroxyacylglutathione hydrolase, mitochondrial (Q6P963) Evalue: 1e-68	430	15 (7)	37.8	217	1.4	2.21	3.76
contig23276:205:1035:1 similar to Haloacid dehalogenase-like hydrolase domain-containing protein 2 (Q5BJJ5) Evalue: 1e-77	328	9 (6)	37.7	276	2.6	1.16	1.05
contig20960:73:1833:1 similar to Ectonucleoside triphosphate diphosphohydrolase 1 (P97687) Evalue: 4e-78	174	4	6.1	586	0.45	0.26	0.1
contig20078:131:626:2 similar to Platelet-activating factor acetylhydrolase IB subunit beta (Q5R4G2) Evalue: 2e-42	167	4	26.1	165	0.38	0.91	0.38
contig04693:206:1270:2 similar to Ester hydrolase C11orf54 homolog (Q6NWE0) Evalue: 6e-68	273	6	23.7	354	0.27	0.62	0.38
contig02919:1:1204:2 similar to Leukotriene A-4 hydrolase (Q3SZH7) Evalue: 3e-130	394	8	21.5	400	0.41	1.14	0.51
contig02433:240:1799:3 similar to Ectonucleoside triphosphate diphosphohydrolase 3 (O75355) Evalue: 2e-59	420	11 (7)	18.1	519		0.54	0.92

contig00596:1:659:3 similar to Peptidyl-tRNA hydrolase 2, mitochondrial (Q3ZBL5) Evalue: 7e-33	55	1	5.5	218			0.28
contig00207:248:982:2 similar to GTP cyclohydrolase 1 (P30793) Evalue: 4e-83	115	3	16.4	244		0.53	
contig00187:1:480:1 similar to N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (O94760) Evalue: 5e-19	159	5 (4)	42.8	159		1.62	1.43
<b>Hydratases</b>							
contig22786:127:2175:1 similar to Urocanate hydratase (Q8VC12) Evalue: 0.0	970	22 (17)	34.3	682	0.09	1.22	0.59
contig00053:1:856:2 similar to Enoyl-CoA hydratase, mitochondrial (Q8BH95) Evalue: 2e-96	575	18 (11)	48.6	284	1.98	4.17	3.48
contig02653:162:2573:3 similar to Aconitate hydratase (Q4JVM4) Evalue: 0.0	480	11 (9)	17	804	0.29	0.69	0.68
contig23758:1:2385:1 similar to Aconitate hydratase, mitochondrial (P20004) Evalue: 0.0	1609	47 (28)	50.1	794	0.95	3.39	2.95
contig25236:33:1556:3 similar to Fumarate hydratase 2, chloroplastic (Q9FI53) Evalue: 0.0	554	13 (11)	29	507	0.32	1.75	2.45
contig19076:172:1149:1 similar to Probable enoyl-CoA hydratase echA8 (P64016) Evalue: 9e-22	527	11 (9)	35.7	325	0.31	1.45	0.71
contig09521:127:1221:1 similar to GDP-mannose 4,6 dehydratase (Q8K0C9) Evalue: 8e-158	438	12 (9)	33	364	0.85	1.46	2
contig08166:1:810:1 similar to Methylglutaconyl-CoA hydratase, mitochondrial (Q13825) Evalue: 4e-76	370	10 (8)	32.7	269	0.38	1.37	1.13
contig25136:1595:1893:3 similar to Probable cytoplasmic aconitate hydratase (Q23500) Evalue: 4e-30	77	2	19.4	98		0.72	
contig17658:1:222:1 similar to Putative pterin-4-alpha-carbinolamine dehydratase (Q9TZH6) Evalue: 6e-05	39	1	12.2	74	0.82		
contig04646:117:1208:3 similar to Enoyl-CoA hydratase domain-containing protein 3, mitochondrial (A0PJR5) Evalue: 3e-71	177	4	16	363		0.37	
<b>Autophagy-related proteins</b>							
<b>ATG3 family</b>							
contig00711:83:900:2 similar to Autophagy-related protein 3 (Q5I0S6) Evalue: 6e-88	126	2	12.1	272	0.36	0.34	0.62
contig17724:1:103:2 similar to Autophagy-related protein 3 (Q6PFS7) Evalue: 3e-09	35	1	45.5	33			1.88
contig14555:14:2122:2 similar to Autophagy-related protein 7 (Q641Y5) Evalue: 0.0	145	4	7.5	702		0.18	
<b>Ligases</b>							
<b>ATP-dependent AMP-binding enzyme family.</b>							
contig26103:184:2216:1 similar to Long-chain-fatty-acid--CoA ligase 5 (Q8JZR0) Evalue: 3e-166	1422	51 (23)	45.1	677	1.89	4.19	3.23
contig11933:40:1665:1 similar to Long-chain-fatty-acid--CoA ligase 5 (Q9ULC5) Evalue: 4e-150	137	3	8.5	541	0.28	0.22	0.33
contig22864:1:1248:1 similar to Long-chain-fatty-acid--CoA ligase ACSBG2 (Q5ZKR7) Evalue: 2e-137	136	3	12	415		0.23	0.15
contig17581:1:708:2 similar to Long-chain-fatty-acid--CoA ligase ACSBG2 (Q7ZYC4) Evalue: 6e-36	71	3 (2)	11.5	235		0.27	
contig05457:1:1217:3 similar to Tubulin--tyrosine ligase-like protein 12 (Q3UDE2) Evalue: 1e-74	216	4	15.3	405		0.31	0.22
contig08317:72:2177:3 similar to GMP synthase [glutamine-hydrolyzing] (Q4V7C6) Evalue: 0.0	485	10	20.3	701	0.33	0.44	0.33
contig21376:72:1232:3 similar to E3 ubiquitin-protein ligase Nedd-4 (Q9VVI3) Evalue: 1e-48	79	2	6.2	387	0.16		
contig17893:1:1353:1 similar to E3 ubiquitin-protein ligase HUWE1 (Q7Z6Z7) Evalue: 0.0	37	1	1.8	450		0.12	
contig16624:1:759:2 similar to E3 ubiquitin-protein ligase HUWE1 (Q7TMY8) Evalue: 6e-45	34	1	6.3	252	0.36		
contig02638:1:1046:3 similar to E3 ubiquitin-protein ligase UBR5 (O95071) Evalue: 2e-55	35	1	1.7	348	0.08		0.08
contig27962:63:1574:3 similar to Glutamate--cysteine ligase catalytic subunit (P48506) Evalue: 6e-120	70	2	5.6	503		0.12	0.39
contig24640:1:1499:3 similar to Glutamate--cysteine ligase catalytic subunit (P48506) Evalue: 7e-128	172	4	9	498		0.25	
contig21482:1:542:2 similar to Glutamate--cysteine ligase catalytic subunit (P97494) Evalue: 2e-33	56	1	8.3	180		0.32	
contig20413:138:508:3 similar to Glutamate--cysteine ligase (Q9W3K5) Evalue: 1e-31	241	4	39	123		1.35	0.9
contig05957:1:1687:2 similar to Glutamate--cysteine ligase catalytic subunit (P97494) Evalue: 0.0	353	9 (8)	17.8	561			0.41
contig02105:90:857:3 similar to Glutamate--cysteine ligase regulatory subunit (P48508) Evalue: 9e-40	702	17 (11)	56.5	255	2.14	4.7	5.66
contig27122:1:258:1 similar to Phosphoribosylformylglycinamide cyclo-ligase, chloroplastic (Q05728) Evalue: 9e-07	183	3	36.5	85	1.57		
contig25822:42:1055:3 similar to Probable succinyl-CoA ligase [GDP-forming] subunit alpha, mitochondrial (P53596) Evalue: 2e-110	460	16 (8)	40.9	337	3.06	2.85	2.75
contig24470:1:155:3 similar to Succinyl-CoA ligase [ADP-forming] subunit beta (A7HT39) Evalue: 1e-09	151	3 (2)	50	50	5.25	7.63	5.25

contig22681:1:150:2 similar to Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial (Q9Z2I8) Evaluate: 6e-14	50	1	18.4	49		1.4	0.7
contig03691:1:1595:3 similar to Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial (Q9Z2I9) Evaluate: 3e-155	863	16 (14)	33.6	530	0.11	1.88	1.69
contig26159:38:948:2 similar to Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial (Q9Z2I8) Evaluate: 1e-80	788	17 (12)	49.8	303	2.82	4.68	4.77
<b>Cell adhesion</b>							
<b>vinculin/alpha-catenin family.</b>							
contig11660:1:535:2 similar to Vinculin (O46037) Evaluate: 1e-40	216	6 (5)	23.6	178	0.54	3.1	2.47
contig10486:1:542:2 similar to Vinculin (Q17162) Evaluate: 4e-44	111	3	15.6	180		1.1	0.36
contig20949:1:294:2 similar to Vinculin (O46037) Evaluate: 9e-36	116	2	23.7	97		1.66	
<b>beta-catenin family.</b>							
contig04399:881:2524:2 similar to Catenin beta (P35224) Evaluate: 1e-55	96	2	3.5	547	0.11	0.11	
contig04399:82:792:1 similar to Catenin beta (P26233) Evaluate: 8e-37	164	3	16.9	236	0.44		
<b>Isomerases</b>							
contig27355:1:743:3 similar to Probable protein disulfide-isomerase A6 (Q11067) Evaluate: 2e-57	429	10 (7)	39.8	246	1.25	1.91	1.91
contig26565:106:1698:1 similar to Protein disulfide-isomerase 2 (Q17770) Evaluate: 6e-144	2450	138 (40)	70.4	530	14.61	36.48	30.18
contig21701:200:1894:2 similar to Protein disulfide-isomerase A3 (P38657) Evaluate: 1e-134	1302	44 (21)	45	564	4.88	6.06	7.48
contig10439:1:711:3 similar to Probable protein disulfide-isomerase A4 (P34329) Evaluate: 8e-07	70	2	10.6	236		0.88	0.26
contig01091:1:706:3 similar to Putative protein disulfide-isomerase C1F5.02 (Q10057) Evaluate: 6e-05	268	6	32.1	234		1.8	0.68
contig00103:97:441:1 similar to Protein disulfide-isomerase A6 (Q63081) Evaluate: 9e-26	255	4 (3)	33.3	114	2.31	2.13	0.88
contig28288:1:1091:3 similar to Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial (Q62651) Evaluate: 5e-81	304	9 (7)	21.8	363	0.43	0.72	0.72
contig03284:1:276:1 similar to Peptidyl-prolyl cis-trans isomerase, mitochondrial (P30405) Evaluate: 1e-36	140	4	50.5	91	9.05	2.34	2.34
contig26555:1:191:3 similar to Peptidyl-prolyl cis-trans isomerase (P25007) Evaluate: 1e-25	102	4 (3)	28.6	63	4.98	2.49	2.49
contig24216:1:620:3 similar to Peptidyl-prolyl cis-trans isomerase (P25007) Evaluate: 1e-75	373	12 (7)	41.5	205	16.32	3.18	2.29
contig18919:1:514:2 similar to Peptidyl-prolyl cis-trans isomerase B (P24369) Evaluate: 3e-57	862	24 (12)	69.4	170	11.75	17.17	11.11
contig00459:180:977:3 similar to Peptidyl-prolyl cis-trans isomerase-like 3 (Q9H2H8) Evaluate: 9e-60	36	2 (1)	2.6	265	1.21	0.77	0.67
contig19225:1:421:1 similar to Peptidyl-prolyl cis-trans isomerase (P25007) Evaluate: 4e-25	144	4 (3)	21.4	140		0.76	0.76
contig02949:47:615:2 similar to Peptidyl-prolyl cis-trans isomerase (P14088) Evaluate: 4e-33	95	1	7.4	189		0.32	0.32
contig06340:1:435:1 similar to Peptidyl-prolyl cis-trans isomerase (P54985) Evaluate: 6e-44	101	4 (3)	20.7	145			0.76
contig00835:1:436:2 similar to Peptidyl-prolyl cis-trans isomerase (P54985) Evaluate: 4e-42	182	5 (4)	33.8	145			1.16
contig01672:1:582:1 similar to Peptidyl-prolyl cis-trans isomerase-like 1 (Q9D0W5) Evaluate: 7e-56	135	2	17.1	193	0.34		
contig18627:1:245:3 similar to Peptidyl-prolyl cis-trans isomerase NIMA-interacting 4 (Q6P4K8) Evaluate: 7e-33	60	2	45	80	0.94		
contig02372:1:427:2 similar to Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Q9QUR7) Evaluate: 3e-46	28	1	7.1	141	0.42		
contig26282:31:442:1 similar to Ribose-5-phosphate isomerase (Q551C2) Evaluate: 6e-20	210	3	29.9	137	1.07	1.07	0.84
contig24083:75:995:3 similar to Triosephosphate isomerase (B0BM40) Evaluate: 1e-90	771	17 (10)	47.4	306	1.86	4.18	5.04
contig23818:1:1081:2 similar to Glucose-6-phosphate isomerase (P08059) Evaluate: 8e-146	613	16 (10)	47.4	359	0.45	2.01	1.27
contig23424:1:726:2 similar to Glucose-6-phosphate isomerase (Q4R591) Evaluate: 1e-63	543	13 (12)	54.8	241	0.71	3.41	1.84
contig19002:1:528:1 similar to Peroxisomal 3,2-trans-enoyl-CoA isomerase (Q9WUR2) Evaluate: 9e-29	182	3	18.2	176	0.77	0.97	1.47
contig17652:1:612:1 similar to Peroxisomal 3,2-trans-enoyl-CoA isomerase (O75521) Evaluate: 1e-57	171	3	21.2	203	1.04	1.04	0.84
contig17585:1:663:1 similar to Glucosamine-6-phosphate isomerase 2 (A4IHW6) Evaluate: 7e-106	275	8 (6)	34.1	220	0.59	1.39	1.74
contig03157:114:302:3 similar to Glucosamine-6-phosphate isomerase (Q9VMP9) Evaluate: 3e-23	124	3 (2)	44.4	63	0.96	1.2	1.2
contig07505:111:1223:3 similar to Methylthioribose-1-phosphate isomerase (Q4FZP2) Evaluate: 3e-108	172	6 (5)	15.1	370	0.26	0.48	0.37
contig11180:674:2278:2 similar to DNA topoisomerase 1 (P11387) Evaluate: 0.0	63	2	3	534	0.11		

contig03799:257:1534:2 similar to Mannose-6-phosphate isomerase (Q3SZIO) Evalue: 2e-86	718	17 (14)	39.1	425	0.36	2.22	1.86
<b>Other proteins</b>							
<b>Calponin family</b>							
contig24510:145:671:1 similar to Myophilin (Q24799) Evalue: 4e-31	505	10 (9)	62.9	175	0.88	5.3	5.13
contig18848:1219:1956:1 similar to Myophilin (Q24799) Evalue: 5e-44	668	25 (10)	55.9	245	2.25	6.64	4.95
contig07731:191:609:2 similar to Myophilin (Q24799) Evalue: 1e-21	114	4 (3)	23	139	5.55	1.29	0.7
<b>Piwi protein family</b>							
contig22518:275:3181:2 similar to Piwi-like protein 1 (Q8UVX0) Evalue: 5e-94	612	15 (14)	16.6	968	0.68		
contig19037:1:694:2 similar to Piwi-like protein 1 (Q8UVX0) Evalue: 3e-37	188	3	14.8	230	0.42		
contig17723:1:394:1 similar to Piwi-like protein 2 (A8KBF3) Evalue: 3e-11	129	2	19.8	131	0.75		
contig06808:1:690:1 similar to Piwi-like protein 1 (Q96J94) Evalue: 3e-47	148	4	17.9	229	0.63		
contig02272:1:1240:2 similar to Piwi-like protein 1 (Q8UVX0) Evalue: 7e-48	542	10 (9)	28.8	413	0.96		
<b>VMO1 protein family</b>							
contig27472:84:670:3 similar to Vitelline membrane outer layer protein 1 (P41366) Evalue: 5e-18	288	7 (3)	26.7	195		1.94	1.56
contig27175:1:1068:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 1e-19	136	3	8.5	355		0.54	0.34
contig12096:1:1521:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-12	574	12 (10)	25.6	507		1.3	0.66
contig01745:31:1008:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 1e-21	661	16 (11)	36.5	326		2.27	4.16
contig01462:37:1185:1 similar to Vitelline membrane outer layer protein 1 homolog (Q7Z5L0) Evalue: 3e-23	1080	48 (17)	58.4	382	0.57	14.7	12.4
contig12095:1:708:1  hypothetical protein Y9C9A (NP_500684.1) Evalue: 2e-09	323	5	33.2	235		1.07	1.65
contig24625:236:982:2 similar to 14-3-3 protein zeta (Q2F637) Evalue: 2e-123	1096	84 (16)	67.7	248	21.14	17.71	25.58
contig11489:1:626:3 similar to 14-3-3 protein 8 (P93213) Evalue: 2e-58	93	2	11.6	207			0.3
contig00699:168:944:3 similar to 14-3-3-like protein D (Q96453) Evalue: 9e-84	577	30 (10)	42.2	258	5.69	2.99	3.18
contig25959:606:1558:3 similar to Annexin A6 (P51901) Evalue: 2e-70	597	17 (14)	55.2	317		2.87	3.31
contig17838:1:476:2 similar to Annexin A6 (P79134) Evalue: 2e-08	169	6 (3)	22.2	158	0.41	2.37	3.27
contig03812:575:1582:2 similar to Annexin-B12 (P26256) Evalue: 7e-85	378	6	21.2	335		0.75	0.66
contig00947:1:818:3 similar to Annexin A11 (P33477) Evalue: 6e-48	1301	52 (22)	78.6	271	1.44	29.14	30.15
contig17491:1:316:2 similar to LanC-like protein 2 (Q9JJK2) Evalue: 5e-38	124	2	24	104	0.66	0.66	
contig01005:123:735:3 similar to LanC-like protein 1 (O43813) Evalue: 7e-34	173	3	26	204	0.49	0.49	0.7
contig00024:1:294:2 similar to LanC-like protein 2 (Q9JJK2) Evalue: 1e-15	74	2	34	97		0.7	
contig20070:1:1807:2 similar to Clathrin heavy chain (P25870) Evalue: 0.0	1126	23 (19)	38.2	602	2.28	3.3	2.48
contig19825:1:349:1 similar to Clathrin heavy chain 1 (P49951) Evalue: 2e-44	210	4 (3)	45.7	116	3.42	2.37	2.1
contig19303:2463:3173:3 similar to Clathrin light chain A (O08585) Evalue: 2e-26	122	2	8.9	236		0.25	
contig22755:88:1314:1 similar to Eukaryotic initiation factor 4A (P27639) Evalue: 4e-136	208	6 (3)	7.4	408	0.46	0.46	0.74
contig00124:1:1376:3 similar to Eukaryotic initiation factor 4A-II (Q8JFP1) Evalue: 2e-166	1504	64 (22)	64.3	457	10.64	11.24	8.44
contig28087:1:1105:2 similar to Cytosolic Fe-S cluster assembly factor nubp1 (Q6P298) Evalue: 2e-126	39	1	2.5	367	0.17	0.24	0.16
contig17703:1:972:1 similar to Cytosolic Fe-S cluster assembly factor NUBP2 (Q5ZKV4) Evalue: 8e-92	174	3	17.6	323	0.18	0.39	0.3
contig09546:228:782:3 similar to Calnexin-2 (P45961) Evalue: 2e-19	177	4	33.2	184		0.82	1.11
contig09257:1:1032:1 similar to Zinc finger protein 622 (Q969S3) Evalue: 7e-36	155	4 (3)	15.7	343	0.38	0.38	0.28
contig23157:49:1047:1 similar to Protein NipSnap (Q9VXK0) Evalue: 5e-68	421	12 (8)	33.1	332	0.51	1.19	1.19
contig24260:85:1125:1 similar to Replication factor C subunit 5 (P40937) Evalue: 1e-107	169	2	8.7	346	0.17		
contig23464:1:469:1 similar to Replication factor C subunit 2 (Q641W4) Evalue: 2e-66	224	4	32.7	156	1.01		

## Supplementary

contig15523:501:1112:3 similar to Replication factor C subunit 3 (Q2TBV1) Evalue: 4e-70	125	3 (2)	11.3	203	0.3			
contig02922:1:981:1 similar to Replication factor C subunit 4 (P35249) Evalue: 1e-106	73	2	5.8	326	0.54	0.19	0.19	
contig02922:1:981:1 similar to Replication factor C subunit 4 (P35249) Evalue: 1e-106	168	5 (4)	15.3	326				
contig02922:1:981:1 similar to Replication factor C subunit 4 (P35249) Evalue: 1e-106	57	2	9.2	326				
contig11183:1:856:3 similar to Golgi apparatus protein 1 (Q92896) Evalue: 7e-44	165	3	19	284	0.47	0.66		
contig08693:1:222:1 similar to Golgi apparatus protein 1 (Q02391) Evalue: 8e-06	82	2	27	74	1.68	0.93	0.93	
contig02007:24:1118:3 similar to Golgi apparatus protein 1 (Q92896) Evalue: 2e-61	102	2	6.9	364	0.25	0.24	0.34	
contig28336:1:110:2 similar to Calbindin-32 (P41044) Evalue: 0.001	94	2	50	36		2.29	2.29	
contig24297:1:452:3 similar to Calbindin-32 (P41044) Evalue: 3e-14	379	7 (5)	51	149		2.13	2.33	
contig24154:38:379:2 similar to Calbindin-32 (P41044) Evalue: 1e-04	200	5 (4)	47.8	113		1.57	1.03	
contig19771:1:769:2 similar to Calbindin-32 (P41044) Evalue: 2e-40	248	5	24.7	255	0.52	1.38	1.97	
contig18616:1:460:2 similar to Calbindin-32 (P41044) Evalue: 2e-15	385	8 (7)	62.5	152	0.44	2.79	1.99	
contig09211:1:109:1023:1 similar to Calbindin-32 (P41044) Evalue: 1e-32	559	13 (10)	35.9	304		1.74	2.11	
contig27864:1:775:2 similar to Sarcoplasmic calcium-binding protein, beta chain (P02635) Evalue: 5e-12	699	20 (13)	51	257		4.54	2.57	
contig03263:1:1127:3 similar to Gelsolin (Q07171) Evalue: 3e-52	885	29 (16)	39.3	374	0.45	3.96	3.75	
contig27035:1:393:1 similar to Carboxylesterase 2 (O00748) Evalue: 6e-26	486	18 (8)	77.1	131	0.51	12.41	7.23	
contig25473:16:285:1 similar to Liver carboxylesterase 1 (P10959) Evalue: 1e-05	39	1	8.9	90		1.8	3.14	
contig25471:1:394:1 similar to Liver carboxylesterase (Q29550) Evalue: 3e-22	309	7 (5)	42.7	131		4.98	4.29	
contig20064:50:1729:2 similar to Vacuolar protein sorting-associated protein 45 (P97390) Evalue: 5e-167	63	2	3.8	560		0.1		
contig19989:292:1617:1 similar to Vacuolar protein sorting-associated protein 4B (O75351) Evalue: 7e-180	304	6 (5)	18.1	441	0.52	0.54	0.45	
contig17918:874:2442:1 similar to Vacuolar protein sorting-associated protein 35 (Q9EQH3) Evalue: 2e-161	247	4	10.2	522	0.31	0.24	0.17	
contig10841:1:1689:1 similar to Vacuolar protein sorting-associated protein 13C (Q709C8) Evalue: 9e-76	201	4	7.8	563	0.42	0.54	0.37	
contig03196:1:1635:1 similar to Vacuolar protein sorting-associated protein 13A (Q96RL7) Evalue: 9e-75	100	2	4.4	544	0.37	0.16	0.11	
contig01644:1:968:1 similar to Vacuolar protein sorting-associated protein 13C (Q8BX70) Evalue: 2e-09	428	9 (7)	33.2	322	2.59	1.88	0.97	
contig03368:1:708:1 similar to Vacuolar protein sorting-associated protein 13C (Q8BX70) Evalue: 1e-17	65	2	9.4	235	1.71	0.27		
contig17918:63:830:3 similar to Vacuolar protein sorting-associated protein 35 (Q96QK1) Evalue: 1e-82	184	3	11.8	255	0.24	0.38		
contig17506:1:300:1 similar to Vacuolar protein sorting-associated protein 29 (B2RZ78) Evalue: 1e-25	122	2	23	100	0.71			
contig00019:121:386:1 similar to Nascent polypeptide-associated complex subunit alpha (Q94518) Evalue: 2e-06	104	3 (1)	25	88	1.72	1.22	1.22	
contig20358:1:418:2 similar to Nascent polypeptide-associated complex subunit alpha (Q94518) Evalue: 2e-17	316	6 (5)	49.3	138	7.21	5.85	4.7	
contig20315:132:1091:3 similar to Malectin (A9C3P0) Evalue: 2e-45	453	9 (8)	37.3	319	1.04	1.57	0.99	
contig00142:113:1300:2 similar to Stomatatin-like protein 2 (Q99JB2) Evalue: 3e-115	362	9 (7)	22.8	395	0.54	0.79	0.66	
contig27883:1:341:3 similar to Vesicle-associated membrane protein 3 (Q4R8T0) Evalue: 5e-11	99	2	23.2	112	1.33	0.89	1.33	
contig26840:156:980:3 similar to Vesicle-associated membrane protein-associated protein B (Q9Z269) Evalue: 6e-40	368	15 (7)	30.3	274	2.23	2.6	2.28	
contig03446:388:1608:1 similar to Kynureninase (Q1DDU5) Evalue: 6e-104	280	6 (5)	20.7	406		0.41	0.32	
contig03471:1:1293:1 similar to Reticulon-4-interacting protein 1, mitochondrial (Q0VC50) Evalue: 3e-71	364	8 (7)	17.4	430	0.49	0.66	0.7	
contig25841:75:1310:3 similar to Reticulon-4 (Q9JK11) Evalue: 9e-27	557	14 (12)	35.9	412	3.52	2.66	3.68	
contig25802:1:1389:1 similar to Enolase (Q27527) Evalue: 0.0	1786	62 (24)	68.8	462	3.1	10.05	7.51	
contig18035:30:1538:3 similar to Mitochondrial enolase superfamily member 1 (Q6INX4) Evalue: 6e-171	435	10 (9)	24.5	502	0.4	0.78	0.81	
contig22522:136:803:1 similar to Prohibitin-2 (Q5XIH7) Evalue: 1e-84	423	18 (8)	41	222	6.76	8.88	10.31	
contig00327:1:382:1 similar to Sarcalumenin (Q90577) Evalue: 2e-12	72	3 (2)	19.7	127		2.13	1.44	
contig00327:1:382:1 similar to Sarcalumenin (Q90577) Evalue: 2e-12	89	4 (2)	19.7	127				



## Supplementary

contig17643:1:773:3 similar to Sarcalumenin (Q7TQ48) Evalue: 2e-39	405	10 (9)	44.9	256		2.16	1.29
contig07287:1:1127:3 similar to Sarcalumenin (P13666) Evalue: 2e-05	293	5	21.1	374	0.53		
contig07237:1:771:1 similar to Leucine-rich repeat-containing protein 55 (Q6ZSA7) Evalue: 4e-12	354	7 (6)	34	256	0.38	1.29	0.72
contig20045:1:1552:2 similar to Leucine-rich repeat-containing protein 47 (Q505F5) Evalue: 2e-79	528	10	25.4	516	0.25	0.74	0.74
contig03483:1:981:1 similar to Leucine-rich repeat-containing protein 57 (Q6INV3) Evalue: 2e-61	54	2	6.4	326	0.18		
contig25403:158:1825:2 similar to Leucine-rich repeat-containing protein 15 (Q80X72) Evalue: 4e-19	85	2	4.3	555		0.11	
contig17629:1:1393:2 similar to Leucine-rich repeat-containing protein 15 (Q8TF66) Evalue: 8e-19	117	2	8.2	463		0.19	
contig09305:1:1800:1 No distinct annotation Best-hit: Leucine-rich repeat-containing protein 15 (Q80X72) Evalue: 1e-26	203	6 (4)	9.2	599		0.32	0.27
contig22416:76:1762:1 similar to Collagen alpha-1(XII) chain (Q60847) Evalue: 6e-26	120	3	5	562		0.38	0.22
contig09152:1:720:1 similar to Collagen alpha-1(IV) chain (P02463) Evalue: 9e-82	184	4	13.8	239		0.97	0.76
contig05223:1:537:1 similar to Collagen alpha-2(IV) chain (P27393) Evalue: 2e-88	194	6 (4)	43.3	178		2.3	1.85
contig21773:1:459:3 similar to Ankyrin-3 (Q12955) Evalue: 3e-53	65	1	5.9	152		0.84	0.67
contig16520:66:1137:3 similar to Ankyrin-2 (Q01484) Evalue: 3e-93	385	6	22.4	357		1.58	1.15
contig12023:1:213:1 similar to Ankyrin-2 (Q01484) Evalue: 2e-18	55	1	14.3	70		2.25	0.9
contig02990:1460:2158:1 similar to Ankyrin repeat domain-containing protein 39 (Q9D2X0) Evalue: 9e-12	228	5	20.3	232	0.28	0.84	0.44
contig26127:1:557:3 similar to Fatty acid-binding protein, adipocyte (O97788) Evalue: 7e-21	234	5 (4)	26.1	184	24.47	2.01	1.15
contig03040:1:420:1 similar to Probable fatty acid-binding protein ENSP00000353650 homolog (Q9DAK4) Evalue: 2e-13	97	2	16.5	139	0.48	0.92	0.66
contig22343:1:591:1 similar to Tumor protein D52 (P55327) Evalue: 1e-08	346	8 (7)	30.1	196		2.58	2.38
contig02656:147:559:3 similar to Tumor protein D52 (Q62393) Evalue: 2e-15	65	1	9.5	137			0.25
contig25515:212:1264:2 similar to Placental protein 11 (P21128) Evalue: 1e-25	142	2	10.3	350	0.17	0.17	0.38
contig08099:115:1539:1 similar to RuvB-like 2 (Q9DE27) Evalue: 7e-171	107	2	5.7	474	0.97	0.13	0.2
contig07984:1:1474:2 similar to RuvB-like 1 (P60123) Evalue: 0.0	190	3	9.2	490	0.69	0.19	0.34
contig04281:105:559:3 similar to Reticulocalbin-2 (Q62703) Evalue: 1e-06	83	2	18.5	151		0.63	0.4
contig04154:1:1031:2 similar to Apoptosis-inducing factor 1, mitochondrial (O95831) Evalue: 3e-88	224	8 (5)	14.6	343	0.28	0.48	0.51
contig19757:390:1970:3 similar to Apoptosis-inducing factor 3 (Q96NN9) Evalue: 6e-106	67	2	4.8	526			0.11
contig24169:185:1633:2 similar to LIM domain-binding protein 3 (Q9JKS4) Evalue: 3e-06	494	11 (10)	28.6	482		1.98	2.35
contig08085:1:1097:2 similar to Lethal(2) giant larvae protein homolog 2 (Q7SZE3) Evalue: 1e-18	311	4	21.1	365	0.79		
contig27917:1:308:2 similar to Charged multivesicular body protein 4b (Q5XGW6) Evalue: 1e-38	210	3	39.2	102	1.16	1.16	1.16
contig27900:171:2096:3 similar to Sec1 family domain-containing protein 1 (Q62991) Evalue: 8e-178	115	2	4.2	641		0.09	0.19
contig27739:1:867:1 similar to Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial (Q5XIT9) Evalue: 9e-132	102	2	10.1	288		0.22	
contig27638:1:193:2 similar to Putative odorant-binding protein A5 (P54185) Evalue: 2e-04	65	1	14.1	64			0.96
contig27637:183:565:3 similar to NMDA receptor-regulated 1-like protein (Q6N069) Evalue: 1e-45	79	3 (2)	15	127		0.86	
contig27607:1:319:2 similar to DDRGK domain-containing protein 1 (Q80WW9) Evalue: 4e-29	156	2	29.5	105	1.12	0.93	1.12
contig27253:1:1078:2 similar to Ribonucleoside-diphosphate reductase large subunit (P07742) Evalue: 3e-142	213	4	15.4	358	0.37		
contig27243:1:308:1 similar to High mobility group protein DSP1 (Q24537) Evalue: 3e-19	95	1	12.7	102	0.58		
contig27214:2451:3041:3 similar to Density-regulated protein (Q5ZJ39) Evalue: 1e-32	138	3 (2)	14.3	196			0.33
contig27214:1:2035:2 similar to Replication protein A 70 kDa DNA-binding subunit (P27694) Evalue: 9e-117	99	2	3.8	677	0.29	0.09	0.14
contig27107:1:312:1 similar to Palmitoyl-protein thioesterase 1 (P45479) Evalue: 3e-18	83	1	14.6	103	0.62	0.54	0.62
contig27098:1:313:2 similar to Staphylococcal nuclease domain-containing protein 1 (Q66X93) Evalue: 2e-16	234	5	47.6	103	1.69	6.26	1.94
contig27060:49:404:1 similar to Protein FAM136A (Q6PBU0) Evalue: 2e-08	124	1	11.9	118	0.5		
contig27059:1:452:3 similar to Calcium-binding mitochondrial carrier protein Aralar1 (Q5RBC8) Evalue: 4e-41	91	4 (2)	16.8	149		0.45	0.75

## Supplementary

contig27034:95:808:2 similar to Phytanoyl-CoA dioxygenase domain-containing protein 1 (Q5U3U0) Evalue: 2e-53	267	5 (4)	24.8	238	0.42	0.8	0.6
contig27028:1:974:3 similar to Adenosine deaminase (B2T672) Evalue: 3e-88	290	6 (5)	27.9	323		1.06	0.78
contig27027:151:1464:1 similar to Leucine-rich repeat neuronal protein 2 (O75325) Evalue: 3e-14	63	2	5.5	437			0.13
contig26984:100:861:1 similar to Phosducin-like protein 3 (Q5RB77) Evalue: 2e-36	92	2	15.4	253			0.24
contig26801:1:1803:1 similar to Pesticidal crystal protein cry8Ba (Q45705) Evalue: 4e-05	925	23 (18)	35.8	600	4.03	2.18	1.79
contig26724:170:466:2 similar to Mitochondrial 2-oxoglutarate/malate carrier protein (P22292) Evalue: 1e-17	78	2	18.2	99		0.76	3.46
contig26723:320:876:2 similar to Tubulin polymerization-promoting protein family member 3 (Q9CRB6) Evalue: 9e-22	51	2	17.8	185			0.16
contig26680:1:265:2 similar to Apolipoprotein D (Q8SPI0) Evalue: 6e-10	228	4	48.3	87		3.01	1.58
contig26628:1:159:1 similar to Fibrinogen alpha chain (P06399) Evalue: 2e-06	63	1	36.5	52		1.14	1.45
contig26623:1:1352:3 similar to Histone deacetylase 6 (Q9Z2V5) Evalue: 1e-77	73	2	5.3	449		0.13	
contig26596:231:851:3 similar to Calcium-binding mitochondrial carrier protein Aralar1 (Q9VA73) Evalue: 1e-70	142	3	15.5	207		0.5	0.97
contig26529:1:206:2 similar to Phosphatidylethanolamine-binding protein homolog F40A3.3 (O16264) Evalue: 6e-08	69	2	33.8	68		1.11	0.9
contig26487:1:266:3 similar to ADP-ribosylation factor-like protein 8A (Q96BM9) Evalue: 9e-32	97	4 (2)	20.7	87	1.12		0.78
contig26430:79:2237:1 similar to General vesicular transport factor p115 (Q9Z1Z0) Evalue: 3e-135	163	2	4.3	719		0.08	0.13
contig26406:1:355:1 similar to WD and tetratricopeptide repeats protein 1 (Q80ZK9) Evalue: 3e-08	29	1	5.9	118			0.48
contig26297:1:466:2 similar to U6 snRNA-associated Sm-like protein LSm1 (Q5E9Z8) Evalue: 2e-20	154	2	18.2	154	0.41		
contig26266:1:1086:2 similar to Mitochondrial import inner membrane translocase subunit TIM50 (Q6NWD4) Evalue: 7e-53	175	4 (3)	13.3	361	0.34	0.26	0.46
contig26231:718:1611:1 similar to FMN-dependent NADPH-azoreductase (O07529) Evalue: 3e-09	210	7 (5)	19.2	297	0.21	0.85	0.75
contig26220:64:2079:1 similar to ADP-ribosylation factor-binding protein GGA1 (Q8R0H9) Evalue: 9e-69	371	11 (7)	11.9	671	0.13	0.36	0.3
contig26109:1:438:1 similar to Saxiphilin (P31226) Evalue: 3e-09	106	2	22.6	146		0.45	0.42
contig26057:1:315:1 similar to Proactivator polypeptide (P26779) Evalue: 4e-06	138	2	26	104	0.63		
contig26020:207:1118:3 similar to Tricarboxylate transport protein, mitochondrial (P79110) Evalue: 5e-107	182	5	16.2	303		0.6	0.43
contig25983:1:1099:2 similar to Protein F37C4.5 (O44400) Evalue: 4e-45	64	2	5.8	365		0.17	0.62
contig25982:1:444:1 similar to Complement component 1 Q subcomponent-binding protein, mitochondrial (Q3T0B6) Evalue: 4e-04	159	2	25	148	0.45	0.45	
contig25889:181:1761:1 similar to Insulin-like growth factor-binding protein complex acid labile chain (O02833) Evalue: 2e-23	574	13 (12)	29.7	526		0.92	1.14
contig25812:1:361:2 similar to Gamma-aminobutyric acid receptor-associated protein-like 1 (Q8R3R8) Evalue: 2e-41	87	1	14.3	119	0.48		
contig25787:1:681:1 similar to Aldo-keto reductase family 1 member C1 homolog (Q95JH7) Evalue: 2e-45	372	11 (6)	31	226	0.4	1.44	1.44
contig25782:1:501:1 similar to Probable nuclear transport factor 2 (Q21735) Evalue: 3e-27	175	6 (4)	24.7	166	2.17		
contig25768:1:505:2 similar to Tissue alpha-L-fucosidase (P17164) Evalue: 5e-54	275	8 (4)	25.6	168		1.97	1.58
contig25738:1:1210:2 similar to Protein pelota (P48612) Evalue: 2e-146	63	2	3.5	402		0.15	
contig25715:1:956:1 similar to Glucosylceramidase (Q2KHZ8) Evalue: 5e-93	468	14 (9)	34	318	1.24	3.44	3.72
contig25711:126:978:3 similar to Counting factor associated protein D (Q54TR1) Evalue: 5e-09	153	4	24.3	284	0.87	0.77	0.46
contig25692:1:1571:3 seems to belong to immunoglobulin superfamily. DCC family Best-hit: Netrin receptor DCC (P70211) Evalue: 2e-08	419	15 (6)	25.1	522	0.24	1.53	1.06
contig25690:1:1517:3 similar to Nucleolar protein 58 (Q6DFW4) Evalue: 6e-146	421	8 (6)	16.2	505	0.52	1.42	1.26
contig25667:167:1171:2 similar to Transaldolase (Q9EQS0) Evalue: 5e-125	695	15 (13)	47.9	334	1.82	2.95	1.91
contig25621:1:352:3 similar to Nucleolar protein 56 (Q95K50) Evalue: 2e-45	216	4	52.6	116			1.51
contig25619:1:1072:2 similar to ATP-dependent RNA helicase uap56 (O13792) Evalue: 4e-144	75	2	8.1	356			0.17
contig25578:1:1277:2 similar to Regulator of chromosome condensation (P23800) Evalue: 4e-45	46	1	3.3	425		0.14	0.14
contig25536:434:1090:2 similar to CD9 antigen (P40240) Evalue: 8e-10	106	2	10	219	0.29		

## Supplementary

contig25525:261:1538:3 similar to Charged multivesicular body protein 7 (Q6PBQ2) Evalue: 1e-37	117	2	7	426	0.22	0.14	
contig25519:1:245:2 similar to Fructose-bisphosphate aldolase, muscle type (P53445) Evalue: 3e-36	267	13 (5)	81.5	81	2.52	19.21	24.28
contig25508:124:265:1 similar to Prickle-like protein 3 (O43900) Evalue: 9e-05	106	2	61.7	47		1.78	1.78
contig25500:19:213:1 similar to Glucan endo-1,3-beta-glucosidase A1 (P23903) Evalue: 2e-07	121	2	36.9	65		2.26	1.59
contig25492:120:779:3 similar to 3~-5~ exoribonuclease CSL4 homolog (Q9DAA6) Evalue: 1e-08	65	2	12.3	219		0.3	
contig25466:143:848:2 similar to AP-2 complex subunit mu-1 (Q5ZMP6) Evalue: 2e-117	144	4 (3)	12.3	235	0.42	0.42	0.42
contig25429:1:2086:2 similar to Carbonic anhydrase-related protein (P28651) Evalue: 9e-21	147	3	5.9	694		0.13	
contig25415:1:546:1 similar to Carbonyl reductase [NADPH] 1 (Q28960) Evalue: 2e-28	152	4	34.8	181	0.35	0.82	0.57
contig25401:1:1670:3 similar to Phosphoacetylglucosamine mutase (Q9CYR6) Evalue: 1e-114	103	4 (3)	5.6	555	0.11	0.22	0.23
contig25357:18:845:3 similar to Replication protein A 32 kDa subunit (Q62193) Evalue: 3e-32	82	2	13.1	275	1.83	0.23	
contig25349:144:1106:3 similar to UPF0363 protein C7orf20 (Q7L5D6) Evalue: 1e-10	106	2	8.4	320	0.19		
contig25323:751:1956:1 similar to Basement membrane-specific heparan sulfate proteoglycan core protein (P98160) Evalue: 2e-15	322	7 (6)	18.7	401		1.33	1.1
contig25323:1:684:1 similar to Basement membrane-specific heparan sulfate proteoglycan core protein (Q05793) Evalue: 3e-06	125	5 (2)	10.1	227		1.28	0.85
contig25319:115:678:1 similar to LDLR chaperone MESD (Q5U2R7) Evalue: 6e-42	80	2	15	187	1.12	0.34	0.34
contig25317:1:1128:1 similar to Phosphate carrier protein, mitochondrial (Q8VEM8) Evalue: 2e-133	464	17 (10)	31.2	375	2.31	7.73	7.31
contig25280:381:983:3 similar to Isochorismatase domain-containing protein 1 (Q08C33) Evalue: 1e-43	540	11 (8)	50	200	1.88	3.94	2.3
contig25250:1:832:2 similar to Nidogen-1 (P10493) Evalue: 6e-46	701	20 (10)	51.4	276	0.72	5.42	3.14
contig25239:1:694:2 similar to LETM1 domain-containing protein 1 (Q28EM8) Evalue: 7e-05	120	3	15.7	230		0.42	0.26
contig25221:147:1340:3 similar to DNA damage-binding protein 1 (Q6P6Z0) Evalue: 2e-65	74	2	4	397		0.15	0.14
contig25175:1:1072:1 similar to Oxysterol-binding protein 2 (Q5QNQ6) Evalue: 4e-115	127	3	9.5	357	1.16	0.43	0.93
contig25145:461:1936:2 similar to Spermine oxidase (Q99K82) Evalue: 6e-50	176	4	11.6	491		0.26	0.26
contig25127:343:2520:1 seems to belong to extended synaptotagmin family Best-hit: Extended synaptotagmin-2 (A0FGR8) Evalue: 5e-102	1307	28 (23)	39	725	0.17	1.91	1.15
contig25105:1:1384:2 similar to Putative endonuclease FLJ39025 (Q8N8Q3) Evalue: 3e-41	83	2	7.4	460	0.13		
contig25085:1:1011:1 similar to Sideroflexin-5 (Q925N0) Evalue: 4e-99	139	4	12.5	336		0.5	0.19
contig25079:1:688:2 similar to Trafficking protein particle complex subunit 3 (Q5ZI57) Evalue: 2e-55	88	2	11.8	228	0.89	0.27	
contig25057:129:1106:3 similar to Periostin (Q62009) Evalue: 3e-07	190	5 (4)	16	325	0.51	1.16	1.02
contig25039:1:687:1 similar to Cysteine and histidine-rich domain-containing protein 1 (Q29RL2) Evalue: 6e-54	107	2	11.8	228	0.28		
contig25016:1:984:1 similar to RNA-binding protein 39 (Q5RC80) Evalue: 8e-117	197	5 (4)	14.1	327	0.42		0.09
contig25002:116:650:2 similar to WW domain-binding protein 2 (P97765) Evalue: 5e-27	118	4 (2)	20.8	178	0.55	0.38	0.9
contig24999:254:1354:2 similar to Acid ceramidase (Q17QB3) Evalue: 2e-117	316	9 (8)	23.5	366	0.44	1.29	0.97
contig24996:316:1764:1 similar to Atlastin (Q9VC57) Evalue: 3e-161	342	6	16.6	483	0.12	0.42	0.26
contig24987:1:1419:1 similar to Zinc finger CCCH domain-containing protein 15 homolog (Q28Y69) Evalue: 7e-67	68	2	3.8	472	0.19	0.13	
contig24984:2765:3460:3 similar to FMN-dependent NADPH-azoreductase (O07529) Evalue: 2e-08	481	17 (10)	44.2	231	4.3	6.4	7.69
contig24982:143:460:2 similar to Cyclin-dependent kinases regulatory subunit (A8XMF2) Evalue: 2e-25	137	5 (3)	26.7	105	1.36		
contig24958:198:1241:3 similar to Aldo-keto reductase family 1 member B10 (O60218) Evalue: 4e-70	489	17 (10)	41.8	347		2.33	
contig24958:198:1241:3 similar to Aldo-keto reductase family 1 member B10 (O60218) Evalue: 4e-70	655	19 (13)	50.1	347			3.11
contig24952:872:1265:2 similar to Major facilitator superfamily domain-containing protein 6 (A1DWM3) Evalue: 2e-17	30	2 (1)	5.3	131			0.46
contig24886:88:1362:1 similar to Multidrug resistance-associated protein 1 (Q8HXQ5) Evalue: 2e-80	38	1	2.1	425			0.14
contig24849:397:2201:1 similar to ATP-dependent RNA helicase DDX1 (Q5XH91) Evalue: 0.0	552	9	20.6	601	0.61	0.53	0.6

## Supplementary

contig24847:1:1562:3 similar to Neurobeachin (Q9W4E2) Evalue: 0.0	94	2	5.6	519	0.12		
contig24832:1:919:2 similar to Pentatricopeptide repeat-containing protein At1g79540 (Q9SAJ5) Evalue: 3e-04	113	2	9.8	306	0.2		0.2
contig24788:499:1563:1 similar to Carbonic anhydrase 2 (P00921) Evalue: 4e-38	297	7	28.5	354		0.73	0.37
contig24762:1:2364:1 similar to RNA-binding protein fusilli (Q9BJZ5) Evalue: 8e-117	141	3	4.3	787			0.12
contig24759:298:1047:1 similar to Stromal membrane-associated protein 2 (Q5F413) Evalue: 1e-45	149	4 (3)	13.7	249	0.55		0.55
contig24689:146:1192:2 similar to Protein E(sev)2B (Q6YKA8) Evalue: 1e-81	67	2	6.6	348	0.17		
contig24682:291:2267:3 similar to Dymeclin (Q5ZLW3) Evalue: 5e-126	162	3	5.9	658	0.08	0.13	0.13
contig24669:144:1168:3 similar to Heterochromatin-associated protein MENT (O73790) Evalue: 7e-43	319	5	21.1	341		0.79	0.79
contig24662:158:1477:2 similar to Glutaminase kidney isoform, mitochondrial (P13264) Evalue: 1e-117	86	2	6.6	440			0.13
contig24650:1:1523:3 similar to TOM1-like protein 2 (Q68FJ8) Evalue: 2e-81	417	10 (9)	26.5	506	0.33	0.77	0.77
contig24574:121:702:1 similar to Prefoldin subunit 3 (Q5RCG9) Evalue: 2e-10	133	3	18.1	193	0.48	0.53	
contig24560:149:1054:2 similar to Proliferating cell nuclear antigen (Q3ZBW4) Evalue: 6e-87	260	6	19.9	301	4.06	0.75	1.11
contig24558:28:783:1 similar to Charged multivesicular body protein 1b (Q5ZKX1) Evalue: 6e-55	159	2	9.6	251	0.25	0.5	0.65
contig24540:1:1008:1 similar to Regulator of microtubule dynamics protein 1 (Q4G069) Evalue: 3e-53	532	13 (9)	29	335	1.02	1.27	1.44
contig24527:81:472:3 similar to Fructose-bisphosphate aldolase C (P53448) Evalue: 7e-46	506	14 (10)	68.5	130	9.1	14.53	13.38
contig24526:125:971:2 similar to ADP/ATP translocase 1 (P02722) Evalue: 6e-119	1023	51 (19)	64.9	282	18.38	28.15	25.9
contig24495:1:615:1 similar to Sorting nexin-6 (Q5R613) Evalue: 3e-57	277	6 (5)	27	204	1.49	1.33	1.49
contig24481:1:553:2 similar to UPF0510 protein INM02 (Q3TAS6) Evalue: 7e-10	111	3	19.7	183	0.76		1.16
contig24429:132:902:3 similar to OCIA domain-containing protein 1 (Q28X44) Evalue: 7e-05	174	3	19.5	256	0.24	0.35	0.38
contig24412:1:775:2 similar to Putative defense protein Hdd11-like (Q86RS3) Evalue: 6e-10	56	2	7	257	0.25		
contig24408:233:636:2 similar to MIT domain-containing protein 1 (Q8WV92) Evalue: 8e-17	36	1	10.4	134		0.44	
contig24406:139:1015:1 similar to Protein DDI1 homolog 2 (A2ADY9) Evalue: 9e-40	289	6	30.5	292	0.59	0.64	0.82
contig24382:1:1710:1 similar to Putative mitochondrial inner membrane protein (P91928) Evalue: 1e-41	1007	29 (22)	41.5	569	0.94	2.44	2.07
contig24379:1:644:3 similar to Gamma-interferon-inducible lysosomal thiol reductase (P13284) Evalue: 2e-28	113	2	11.3	213		0.3	0.69
contig24365:121:1860:1 similar to Moesin/ezrin/radixin homolog 1 (Q170J7) Evalue: 0.0	988	29 (19)	34.9	579	2.29	1.83	1.83
contig24364:1:670:2 seems to belong to tango11 family Best-hit: Transport and Golgi organization protein 11 (Q961C9) Evalue: 2e-09	124	3	16.2	222	1.01	0.59	0.29
contig24351:140:1939:2 similar to JmjC domain-containing protein C2orf60 (A2RUC4) Evalue: 9e-05	445	8 (7)	15.5	599		0.65	0.52
contig24350:1:1439:3 similar to 6PF-2-K/Fru-2,6-P2ASE liver/muscle isozymes (Q91309) Evalue: 3e-158	94	1	2.7	478		0.12	
contig24340:1:990:1 similar to Lysine-specific demethylase rbr-2 (Q61T02) Evalue: 7e-15	143	3	8.5	330		0.37	0.56
contig24302:1:694:3 similar to LAMP family protein C20orf103 homolog (A4FV27) Evalue: 2e-10	205	3	17.8	230	0.26	1.14	0.39
contig24300:1167:2504:3 similar to Rab11 family-interacting protein 1 (Q6WKZ4) Evalue: 8e-24	72	2	4	445			0.14
contig24300:1:957:3 similar to S-methyl-5--thioadenosine phosphorylase (Q13126) Evalue: 3e-90	107	2	9.7	319	0.8	0.29	0.29
contig24294:171:1040:3 similar to RING finger protein 170 (Q96K19) Evalue: 3e-44	203	4	12.8	289	0.3	0.45	0.2
contig24293:145:1845:1 similar to Phosphoglucomutase-1 (P00949) Evalue: 0.0	825	19 (14)	42.6	566	0.29	1.56	1.44
contig24257:95:931:2 similar to Toll-interacting protein (Q9H0E2) Evalue: 3e-53	115	3	12.9	278	0.66	0.35	0.22
contig24243:1:509:3 similar to PRELI domain-containing protein 1, mitochondrial (Q32KN9) Evalue: 2e-35	66	2	11.3	168			0.38
contig24242:93:1841:3 similar to Arylsulfatase J (Q8BM89) Evalue: 8e-66	77	2	3.3	582		0.05	
contig24231:1:1586:1 similar to Glutaminase kidney isoform, mitochondrial (P13264) Evalue: 1e-141	43	1	2.1	528		0.12	0.18
contig24215:1:235:2 similar to UPF0027 protein C22orf28 homolog (Q5E9T9) Evalue: 7e-31	183	3	50.6	77	1.84	2.26	1
contig24212:1:1198:2 similar to UDP-N-acetylhexosamine pyrophosphorylase (Q16222) Evalue: 6e-122	177	4 (3)	10.1	398		0.24	0.24
contig24199:214:1227:1 similar to Uncharacterized protein ZK1073.1 (O02485) Evalue: 2e-70	1199	36 (18)	66.5	337	2.49	10.66	9.09

## Supplementary

contig24163:1:1502:3 similar to Insulin-degrading enzyme (Q24K02) Evalue: 2e-100	512	12 (10)	22.8	499	0.71	1.07	0.38
contig24138:1:740:2 similar to cAMP-dependent protein kinase regulatory subunit (P31319) Evalue: 2e-96	122	2	16.3	246		0.26	0.24
contig24113:1:114:1337:3 similar to Ribonucleoside-diphosphate reductase subunit M2 (P31350) Evalue: 4e-142	306	6 (5)	17	407	0.64		
contig23999:1:1855:1 similar to 1,4-alpha-glucan-branching enzyme (Q8NKE1) Evalue: 0.0	423	9 (8)	16.8	618	0.09	0.7	0.42
contig23922:132:1352:3 similar to Trans-2-enoyl-CoA reductase, mitochondrial (Q6GQN8) Evalue: 1e-67	182	3	10.6	406	0.24	0.24	0.33
contig23905:31:253:1 similar to ATP-dependent RNA helicase SUB2 (Q6CH90) Evalue: 3e-17	89	2	29.7	74		1.04	1.04
contig23884:1:1288:2 similar to Eukaryotic peptide chain release factor subunit 1 (Q5U2Q7) Evalue: 0.0	652	12 (10)	30.4	428	1.07	1.3	1.28
contig23880:1:679:2 similar to Cdc42 homolog (P40793) Evalue: 2e-100	182	8 (4)	24	225	1.09	0.98	0.98
contig23841:88:337:1 similar to Trans-2,3-enoyl-CoA reductase (Q9NZ01) Evalue: 4e-04	46	1	8.4	83	0.74		
contig23840:437:1324:2 similar to Surfeit locus protein 4 homolog (Q18864) Evalue: 2e-80	68	1	5.1	295		0.2	0.41
contig23799:1:405:3 similar to Transforming growth factor beta-1-induced transcript 1 protein (Q62219) Evalue: 5e-38	177	3	32.8	134		0.75	0.4
contig23798:406:933:1 similar to Actin-binding Rho-activating protein (Q8N0Z2) Evalue: 8e-26	152	4	29.1	175		0.86	0.76
contig23741:298:1137:1 similar to Pyrroline-5-carboxylate reductase (P54904) Evalue: 5e-61	301	6	26.5	279	0.53	1.13	0.53
contig23727:127:981:1 similar to Glucan endo-1,3-beta-glucosidase A1 (P23903) Evalue: 3e-18	245	6	26.4	284		0.81	0.64
contig23717:62:627:2 similar to Cation-dependent mannose-6-phosphate receptor (P24668) Evalue: 2e-16	38	1	6.4	188	0.33	0.3	
contig23599:188:1027:2 similar to Uncharacterized protein C56G2.4 (Q09288) Evalue: 1e-04	161	3	15.8	279	1.64	0.35	
contig23535:1845:2589:2 similar to Phosphatidylethanolamine-binding protein homolog F40A3.3 (O16264) Evalue: 1e-44	209	5 (4)	21.5	247	0.96	0.68	0.68
contig23512:1:974:3 similar to Chymotrypsinogen B (Q9CR35) Evalue: 5e-22	136	3	13.3	323		0.3	0.39
contig23493:127:855:1 similar to Calcyclin-binding protein (Q3T168) Evalue: 3e-15	133	4 (3)	8.3	242	1.03	0.41	0.77
contig23480:1:1099:2 similar to Mitochondrial carrier homolog 2 (Q791V5) Evalue: 3e-20	518	13 (11)	32.6	365	2.25	3.22	2.45
contig23475:1:1826:3 similar to AMP deaminase 2 (Q02356) Evalue: 0.0	63	2	2.3	607		0.1	
contig23473:1:954:3 similar to Spliceosome RNA helicase BAT1 (Q3T147) Evalue: 6e-119	576	14 (12)	35	317	3.42	2.52	1.59
contig23448:1:450:2 similar to Synaptobrevin homolog YKT6 (Q9CQW1) Evalue: 2e-31	126	3	22.8	149	0.38	0.7	0.7
contig23436:1:555:1 similar to Adapter molecule Crk (Q9XYM0) Evalue: 4e-44	107	3	14.6	185		0.53	0.15
contig23411:2101:2871:1 similar to AH receptor-interacting protein (O08915) Evalue: 2e-57	87	2	10.5	256	0.88	0.34	0.11
contig23327:25:746:1 similar to Fumarylacetoacetase (P25093) Evalue: 8e-88	344	11 (7)	37.1	240	1.09	1.6	2.06
contig23314:1:731:3 similar to Protein I(2)37Cc (P24156) Evalue: 2e-102	640	16 (10)	50.8	242	4.58	4.21	5.57
contig23273:1:455:3 similar to Aldose reductase (P15121) Evalue: 4e-32	160	5 (3)	26.7	150	0.69	0.88	0.42
contig23257:292:1380:1 similar to Adenosine deaminase (B2T672) Evalue: 3e-75	136	2	7.7	362		0.17	0.17
contig23182:1:1368:1 similar to Cytosolic purine 5--nucleotidase (Q6DKB0) Evalue: 5e-137	746	17 (15)	35.7	456	0.64	1.8	1.67
contig23107:1:1647:1 similar to FACT complex subunit SSRP1 (Q08943) Evalue: 4e-141	99	2	3.6	549	0.11		
contig23103:1:858:1 similar to Kynurenine formamidase (Q8Y1D0) Evalue: 4e-04	141	3 (2)	10.1	286			0.21
contig23090:1:1339:2 similar to Hsp90 co-chaperone Cdc37 (Q24276) Evalue: 5e-92	140	3	6.1	445	0.34	0.2	0.2
contig23076:206:778:2 similar to Ribosome maturation protein SBDS (A5D8M6) Evalue: 2e-79	39	1	4.2	191	0.76	0.3	0.86
contig23060:207:773:3 similar to Pituitary tumor-transforming gene 1 protein-interacting protein (Q8R143) Evalue: 6e-14	91	2	9.6	188	0.63		0.3
contig23054:1:721:2 similar to Uncharacterized protein DR_0705 (Q9RWG3) Evalue: 5e-07	130	2	13.8	239		0.26	0.38
contig23047:1:1368:2 similar to Nucleobindin-2 (P80303) Evalue: 1e-68	85	3 (2)	8.4	455	0.2	0.26	0.27
contig23035:1356:1868:2 similar to RhoA activator C11orf59 homolog (Q6P791) Evalue: 9e-15	163	3	29.4	170	0.63		
contig23004:1:974:3 similar to Peptide-N(4)-(N-acetyl-beta-glucosaminy)l asparagine amidase (Q28YQ7) Evalue: 5e-26	476	19 (9)	35.9	323	9.22	2.79	3.72
contig22978:313:1047:1 similar to Uncharacterized protein MJ0783 (Q58193) Evalue: 1e-06	344	8 (6)	31.1	244	1.8		0.42
contig22943:472:2127:1 similar to HEAT repeat-containing protein 7A (Q8NDA8) Evalue: 3e-15	621	14 (11)	30.9	551	0.65	0.78	0.65

## Supplementary

contig22941:272:2386:2 similar to Solute carrier family 15 member 2 (Q9ES07) Evalue: 4e-116	185	4	8	704	0.18	0.09
contig22888:1:1040:3 similar to CDK5RAP3-like protein (Q95SK3) Evalue: 5e-24	337	5	18.6	345	0.51	0.18
contig22870:3192:3947:1 similar to Tensin (Q04205) Evalue: 2e-68	87	2	8	251		0.25
contig22868:1:2146:2 similar to Crooked neck-like protein 1 (Q9BZJ0) Evalue: 0.0	31	1	1.7	714	0.08	
contig22819:124:1136:1 similar to Diphosphomevalonate decarboxylase (Q5U403) Evalue: 1e-86	166	2	13.4	337		0.18
contig22818:184:1353:1 similar to RNA-binding protein MEX3A (A1L020) Evalue: 2e-59	127	3	8.7	389	0.25	
contig22787:52:1620:1 similar to DOMON domain-containing protein CG14681 (Q9VGY6) Evalue: 1e-25	179	3	11.7	522		0.18 0.12
contig22781:181:839:1 similar to Peptide-N(4)-(N-acetyl-beta-glucosaminy)asparagine amidase (Q503I8) Evalue: 4e-11	159	4 (3)	17.4	219	0.45	0.45
contig22773:107:1537:2 similar to Transducin beta-like protein 2 (Q9Y4P3) Evalue: 6e-73	101	2	5	476	0.13	0.12
contig22743:191:874:2 similar to UPF0480 protein C15orf24 homolog (Q5TYV0) Evalue: 5e-19	211	3	19.4	227	0.41	0.45 0.28
contig22731:224:1087:2 similar to Endoplasmic reticulum-Golgi intermediate compartment protein 1 (Q9DC16) Evalue: 1e-97	163	5 (4)	15	287	0.79	1.09 0.68
contig22720:1:938:3 similar to Glycolipid transfer protein domain-containing protein 1 (Q6DBQ8) Evalue: 7e-27	217	4	13.8	311		0.43 0.31
contig22667:278:2746:2 similar to AP-1 complex subunit gamma-1 (Q5R5M2) Evalue: 0.0	172	4	6.1	823	0.15	
contig22667:278:2746:2 similar to AP-1 complex subunit gamma-1 (Q5R5M2) Evalue: 0.0	140	2	2.8	823		0.07
contig22647:1:758:3 similar to Thaumatin-like protein 2 (P83335) Evalue: 3e-17	71	2	6.4	251		0.25
contig22646:1561:2074:2 similar to Calumenin-A (Q6IQP3) Evalue: 5e-29	30	1	4.1	170		0.34
contig22628:1:949:2 similar to Porphobilinogen deaminase (P08397) Evalue: 3e-75	81	2	8.9	315		0.2
contig22568:102:1820:3 similar to ATP-dependent DNA helicase 2 subunit 2 (P27641) Evalue: 1e-58	98	2	7.2	572		0.1
contig22566:554:2173:2 similar to Nephrocystin-3 (Q6AZT7) Evalue: 1e-08	185	3	5.9	540		0.11
contig22541:639:2438:3 similar to Thyrotropin-releasing hormone-degrading ectoenzyme (Q8K093) Evalue: 7e-32	149	4	6.7	599		0.2
contig22427:1:353:3 similar to Transforming growth factor-beta-induced protein ig-h3 (P82198) Evalue: 1e-04	180	7 (3)	19	116	2.64	3.59 4.12
contig22415:1:289:3 similar to Tryptase gamma (Q9NRR2) Evalue: 2e-10	197	7 (4)	52.6	95		3.25 5.01
contig22377:157:2052:1 similar to Epidermal growth factor receptor kinase substrate 8 (Q12929) Evalue: 9e-58	110	3	6.5	631		0.14
contig22364:1:1748:3 similar to Selenoprotein O (Q9BVL4) Evalue: 4e-154	173	4	8.6	581		0.21 0.15
contig22325:365:2581:2 similar to Uncharacterized family 31 glucosidase KIAA1161 (Q6NSJ0) Evalue: 2e-110	995	23 (18)	34	738	1.45	1.39 1.72
contig22309:1:417:3 similar to UDP-glucose 4-epimerase (Q5R8D0) Evalue: 3e-41	250	7 (5)	50	138	0.81	2.27 1.69
contig22299:1:377:2 similar to Nucleolar protein 56 (O00567) Evalue: 7e-57	77	1	10.4	125	1.63	0.48 1.39
contig22256:1:376:2 similar to Pyridoxine-5--phosphate oxidase (Q5E9K3) Evalue: 1e-26	31	1	12.1	124		0.46
contig22240:311:1744:2 similar to Beta-hexosaminidase subunit beta (P49614) Evalue: 5e-62	197	4	9.4	478		0.33 0.13
contig22235:1:368:3 similar to Aromatic-L-amino-acid decarboxylase (P48861) Evalue: 3e-21	61	2	12.4	121		0.54
contig22205:64:1740:1 similar to Lariat debranching enzyme (Q923B1) Evalue: 1e-115	71	2	5	558	0.11	
contig22173:1:1260:1 similar to ATP-dependent DNA helicase 2 subunit 1 (O93257) Evalue: 2e-71	116	2	5.3	419	0.6	0.14 0.14
contig22164:1:1918:2 similar to DNA mismatch repair protein Msh2 (Q5XXB5) Evalue: 3e-114	149	2	5.3	638	0.09	
contig22126:1:390:1 similar to Spartin (Q8R1X6) Evalue: 7e-07	49	1	8.5	129		0.5
contig22109:1:1475:3 similar to Lysosome-associated membrane glycoprotein 1 (P05300) Evalue: 7e-12	361	10 (7)	16.9	490	1.81	2.74 1.9
contig22083:1:941:3 similar to Astacin (P07584) Evalue: 1e-34	98	2	13.5	312		0.4 0.29
contig22067:1:882:1 similar to Aldo-keto reductase family 1 member C23-like protein (Q6W8P9) Evalue: 1e-63	249	8 (6)	23.5	293	0.21	1.07 0.81
contig22065:1:348:1 similar to Probable L-xylulose reductase (Q21929) Evalue: 3e-15	237	4	38.3	115	2.73	1.93 1.71
contig22044:1:1305:1 similar to 3-ketoacyl-CoA thiolase, mitochondrial (Q3TOR7) Evalue: 8e-145	645	18 (11)	35.9	434	2.38	2.16 1.41
contig22037:1:815:3 similar to Leucine-rich repeats and immunoglobulin-like domains protein 2 (O94898) Evalue: 7e-18	65	3 (2)	2.2	270		0.23
contig22023:191:607:2 similar to HIG1 domain family member 1B (Q9P298) Evalue: 2e-07	165	5 (3)	26.8	138	2.26	

## Supplementary

contig21981:1:526:2 similar to Paxillin (Q66H76) Evalue: 2e-24	39	1	5.7	174	0.32		
contig21953:1:167:3 similar to Cofilin/actin-depolymerizing factor homolog (P45594) Evalue: 2e-20	162	6 (3)	44.4	54	10.83	5.86	3.7
contig21925:1:156:1 similar to Complement component 1 Q subcomponent-binding protein, mitochondrial (Q3T0B6) Evalue: 2e-05	98	3 (2)	56.9	51	1.2	2.16	
contig21843:1:313:2 similar to Allergen Asp f 7 (O42799) Evalue: 3e-05	36	2 (1)	11.5	104			0.6
contig21809:1:323:3 similar to Methylmalonyl-CoA mutase, mitochondrial (Q9GK13) Evalue: 2e-42	121	3 (2)	18.7	107		0.66	
contig21658:1:534:1 similar to Cytosolic purine 5--nucleotidase (Q5RA22) Evalue: 6e-23	415	8 (7)	66.7	177		2.16	1.19
contig21639:1:2042:3 similar to Nephrocystin-3 (Q7TNH6) Evalue: 2e-13	473	10	17.1	679		0.5	0.23
contig21609:2391:3334:3 similar to Chromobox protein homolog 2 (P30658) Evalue: 4e-12	143	2	11.2	313			0.2
contig21602:1:1725:1 similar to Far upstream element-binding protein 1 (Q96AE4) Evalue: 2e-53	143	3	5.7	574	0.17	0.17	
contig21546:1:917:3 similar to D-erythrose reductase (Q8JIS3) Evalue: 1e-77	380	8 (7)	28.3	304		1.48	0.94
contig21510:1:1417:2 similar to Putative neutral sphingomyelinase (O45870) Evalue: 2e-64	30	1	1.5	471		0.12	
contig21503:1:335:1 similar to Alpha-glucosidase 2 (Q9F234) Evalue: 7e-06	208	3	45	111		1.28	0.6
contig21503:1:335:1 similar to Alpha-glucosidase 2 (Q9F234) Evalue: 7e-06	127	2	26.1	111			0.24
contig21380:1:778:2 similar to Mitochondrial Rho GTPase 1 (Q2HJF8) Evalue: 5e-17	119	2	10.5	258			0.18
contig21258:77:842:2 similar to Basement membrane-specific heparan sulfate proteoglycan core protein (Q05793) Evalue: 7e-29	100	2	7.8	255		0.84	0.49
contig20826:1:842:2 similar to Translational activator GCN1 (Q92616) Evalue: 4e-27	387	10 (8)	30.7	280	1.5		
contig20824:1:778:3 similar to Staphylococcal nuclease domain-containing protein 1 (Q7KZF4) Evalue: 1e-65	571	15 (11)	53.1	258		5.07	3.48
contig20819:676:858:1 similar to Ribosome maturation protein SBDS (Q5Z1Y4) Evalue: 4e-12	103	2	36.7	60		1.9	1.36
contig20811:97:705:1 similar to Sorting nexin-6 (Q5R613) Evalue: 2e-75	347	8 (7)	53.7	203	1.52	1.52	1.87
contig20802:1:732:1 similar to Mitochondrial dicarboxylate carrier (Q9QZD8) Evalue: 2e-63	156	4	19.8	243		0.59	
contig20765:101:565:2 similar to Kynurenine--oxoglutarate transaminase 3 (Q6YP21) Evalue: 3e-50	129	3	32.9	155	1.79	0.41	0.98
contig20741:96:1172:3 similar to Chymotrypsinogen B (P07338) Evalue: 1e-34	32	1	2.2	358		0.16	
contig20705:1:1481:3 similar to Insulin-like growth factor-binding protein complex acid labile chain (O02833) Evalue: 2e-15	51	1	2.2	492		0.12	0.12
contig20654:1:611:3 similar to Nucleotide-binding protein-like (Q8TB37) Evalue: 3e-80	139	2	15.3	203		0.32	0.52
contig20639:193:1182:1 similar to N(4)-(Beta-N-acetylglucosaminy)-L-asparaginase (O02467) Evalue: 3e-88	139	2	9.4	330	0.29		
contig20539:162:986:3 similar to Carbonyl reductase [NADPH] 3 (O75828) Evalue: 2e-58	226	4	16.8	274		0.52	0.52
contig20349:88:1161:1 similar to mRNA-decapping enzyme 1B (Q8IZD4) Evalue: 3e-11	89	2	9.2	357	0.88	0.18	0.26
contig20312:1:900:1 similar to Multifunctional protein ADE2 (P51583) Evalue: 1e-75	341	5	22.1	299	2.83	0.61	0.21
contig20281:84:2066:3 similar to Beta-galactosidase (Q58D55) Evalue: 2e-142	234	6 (4)	9.5	660		0.19	
contig20273:1:768:1 similar to Tetratricopeptide repeat protein 35-B (Q8AVU9) Evalue: 1e-66	412	10 (7)	39.5	256	0.69	1.08	0.69
contig20202:1:307:2 similar to IST1 homolog (P53990) Evalue: 7e-24	99	2	13.7	102	0.66		
contig20174:1:768:1 similar to Methylmalonyl-CoA mutase, mitochondrial (Q8HXX1) Evalue: 9e-94	211	4	17.6	255		0.57	0.57
contig20143:1:572:3 similar to Phosphomannomutase (Q1W376) Evalue: 1e-70	273	6	30.7	189	1.04	1.88	1.1
contig20074:1:978:1 similar to Protein roadkill (Q9VFP2) Evalue: 2e-26	244	6	19.1	325		0.67	0.29
contig20066:1:1250:3 similar to Lupus La protein homolog A (P28048) Evalue: 1e-22	294	9 (7)	20.4	416	0.72	0.72	1.1
contig20051:1:752:2 similar to Poly [ADP-ribose] polymerase 3 (Q9Y6F1) Evalue: 8e-35	84	2	9.6	250		0.25	
contig20039:1:1066:2 similar to THUMP domain-containing protein 1 (Q24K03) Evalue: 3e-33	61	2	9.3	354			0.17
contig20024:1:210:1 similar to Heterochromatin-associated protein MENT (O73790) Evalue: 3e-07	164	8 (4)	58	69	0.22	10.73	12.7
contig19973:164:2185:2 similar to Anoctamin-10 (Q4V8U5) Evalue: 4e-126	60	2	3.4	673		0.12	0.16
contig19947:1:454:2 similar to Protein LZIC (Q8WZA0) Evalue: 2e-35	54	1	7.3	150		0.38	0.38

## Supplementary

contig19898:1:918:2 similar to DNA replication licensing factor mcm7-A (Q91876) Evalue: 5e-112	81	2	9.2	305	0.2		
contig19891:1872:2780:3 similar to Uncharacterized protein KIAA0090 homolog (Q5ZL00) Evalue: 7e-72	316	6 (5)	26.8	302		0.91	0.31
contig19869:105:422:3 similar to Muscle LIM protein 1 (P53777) Evalue: 8e-22	159	3	49.5	105	13	1.83	0.67
contig19859:232:908:1 similar to Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (Q92538) Evalue: 6e-43	99	3	16.9	225	0.27	0.57	
contig19837:156:992:3 similar to Nitric oxide synthase-interacting protein (Q6P829) Evalue: 7e-78	85	2	8.6	278	0.23		
contig19812:1:498:1 similar to Down syndrome critical region protein 3 homolog (O35075) Evalue: 1e-53	39	1	4.8	165		0.36	
contig19747:1:421:2 similar to Synaptobrevin homolog YKT6 (Q7ZUN8) Evalue: 2e-23	112	4 (3)	20.1	139	1.01		
contig19659:108:1059:3 similar to Protein FAM185A (Q8N0U4) Evalue: 4e-12	98	2	6.9	317		0.2	
contig19569:1:625:2 similar to Uncharacterized protein T19C3.4 (Q10010) Evalue: 5e-35	101	3 (2)	10.6	207	0.44		
contig19554:1:409:2 similar to U6 snRNA-associated Sm-like protein LSm2 (Q9Y333) Evalue: 2e-31	144	6 (3)	28.1	135	1.27		
contig19541:1:386:2 similar to RNA-binding protein Rsf1 (Q24491) Evalue: 2e-08	242	14 (3)	53.1	128	6.58	8.25	8.05
contig19537:1:390:1 similar to Phenylalanine-4-hydroxylase (P00439) Evalue: 1e-42	145	3 (2)	25.6	129	0.85		1.8
contig19515:1:452:3 similar to ATP-dependent RNA helicase WM6 (Q27268) Evalue: 2e-70	157	4 (3)	21.5	149	2.36	1.34	1.65
contig19510:2385:3152:1 similar to Histone deacetylase 10 (Q969S8) Evalue: 3e-08	162	4	28.2	255		0.56	0.25
contig19496:1:1162:2 similar to Peroxisomal N(1)-acetyl-spermine/spermidine oxidase (Q6QHF9) Evalue: 1e-33	211	5	16.6	386		0.44	0.34
contig19457:1:1683:1 similar to Niemann-Pick C1 protein (P56941) Evalue: 7e-175	38	1	2.5	560		0.1	0.1
contig19397:1:527:3 similar to Lysosomal alpha-mannosidase (Q60HE9) Evalue: 6e-31	70	2	14.9	174	0.77	0.91	0.37
contig19357:147:1667:3 similar to Probable ATP-dependent RNA helicase DDX6 (Q0IHV9) Evalue: 0.0	696	14 (13)	35	506	1.18	1.58	1.4
contig19331:102:1439:3 similar to Legumain (Q9R0J8) Evalue: 1e-112	317	7 (6)	22	445		0.99	0.43
contig19230:1:677:3 similar to Nidogen-1 (P14543) Evalue: 1e-20	76	2 (1)	11.2	224		0.41	0.26
contig19203:180:1808:3 similar to Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial (Q8T2J9) Evalue: 0.0	532	8	24.2	542	0.46	1.08	1.23
contig19185:1:694:2 similar to Protein MEMO1 (Q4QQR9) Evalue: 2e-80	85	2	9.1	230	1.17	0.27	0.43
contig19184:1:906:1 similar to Synapse-associated protein 1 (Q9D5V6) Evalue: 1e-37	116	2	9.6	301			0.31
contig19114:1:1606:2 similar to Golgi resident protein GCP60 (Q9H3P7) Evalue: 1e-48	130	2	4.1	534		0.11	
contig19078:1:710:3 similar to Protein canopy homolog 4 (Q8BQ47) Evalue: 5e-26	91	2	11	236	0.38		
contig19065:21:936:3 similar to Beta-ureidopropionase (Q5RBM6) Evalue: 5e-110	67	2	7.2	305	0.2	0.3	
contig19035:11:838:2 similar to F-actin-capping protein subunit beta (Q5R507) Evalue: 2e-129	264	7 (5)	28	275	0.5	0.83	
contig18939:144:776:3 similar to Membrane-associated progesterone receptor component 2 (Q5XIU9) Evalue: 3e-35	215	5	32.9	210	0.49	1.66	1.66
contig18933:1:2374:2 similar to Protocadherin Fat 1 (Q14517) Evalue: 1e-14	504	11	19.2	790		0.45	0.21
contig18923:1:1090:2 similar to Protein TFG (Q92734) Evalue: 1e-20	124	3 (2)	10.2	362	0.6	0.36	0.46
contig18905:252:1415:3 similar to Heterochromatin-associated protein MENT (O73790) Evalue: 7e-57	491	12 (11)	37	387	0.25	1.88	1.62
contig18896:158:1162:2 similar to Small glutamine-rich tetratricopeptide repeat-containing protein beta (Q80W98) Evalue: 6e-38	236	5 (4)	14.3	335	0.6	0.68	0.54
contig18894:41:1108:2 similar to CUE domain-containing protein 1 (Q8R3V6) Evalue: 1e-19	30	1	2	355	0.5	0.24	0.24
contig18840:102:891:3 similar to Transforming growth factor-beta-induced protein ig-h3 (Q15582) Evalue: 4e-09	366	17 (7)	24	263	5.06	5.91	7.02
contig18803:325:990:1 similar to Synaptogyrin-2 (A7E3W5) Evalue: 6e-24	57	2 (1)	5.4	221	0.56	0.56	0.42
contig18759:1:680:3 similar to Extracellular domains-containing protein CG31004 (Q0KH33) Evalue: 2e-32	299	6	31.9	226		1.95	1.5
contig18732:1:1015:2 similar to Carbonyl reductase [NADPH] 1 (P47844) Evalue: 3e-73	393	9 (8)	35.5	338	1.12	1.22	0.96
contig18731:1:341:3 similar to ADP-ribosylation factor-like protein 5A (Q80ZU0) Evalue: 1e-42	58	2	17	112	0.6		
contig18711:1:684:1 similar to Omega-amidase NIT2 (Q28IE5) Evalue: 1e-83	121	2	15	227	0.28		0.44
contig18709:206:705:2 similar to Epididymal secretory protein E1 (P61918) Evalue: 2e-16	161	2	18.7	166	0.4	0.4	0.4



## Supplementary

contig18699:1:560:1 similar to High mobility group protein DSP1 (Q24537) Evaluate: 8e-48	227	4	23.7	186	1.56			
contig18649:1:773:3 similar to CDGSH iron sulfur domain-containing protein 2 homolog (Q9VAM6) Evaluate: 2e-26	65	2	11.7	256	0.22	0.24	0.24	
contig18620:65:748:2 similar to Synaptosomal-associated protein 23 (O70377) Evaluate: 1e-44	240	4	26.4	227	0.62		0.62	
contig18607:216:1205:3 similar to Purine nucleoside phosphorylase (P00491) Evaluate: 1e-90	75	2	6.7	329		0.19		
contig18572:128:715:2 similar to Mitochondrial import inner membrane translocase subunit Tim23 (O14925) Evaluate: 3e-35	141	2	14.9	195	0.67		0.35	
contig18571:1:661:2 similar to Vesicle-trafficking protein SEC22b (Q5ZJW4) Evaluate: 5e-72	237	4	25.1	219	1.11	0.91	0.84	
contig18547:1:337:2 similar to Uncharacterized protein sll1483 (P74615) Evaluate: 5e-06	160	5 (4)	57.7	111	6.86	5.02	6.34	
contig18534:97:1770:1 similar to NEDD8-activating enzyme E1 regulatory subunit (Q13564) Evaluate: 3e-136	157	3	7.4	557	0.16		0.11	
contig18531:1:199:1 similar to Cofilin/actin-depolymerizing factor homolog (P45594) Evaluate: 2e-16	151	5 (2)	57.6	66	4.69	2.12	2.12	
contig18525:64:726:1 similar to Protein alan shepard (B4MM23) Evaluate: 1e-57	86	2	9.5	221	0.66	0.29	0.46	
contig18513:112:668:1 similar to FMN-dependent NADPH-azoreductase (O07529) Evaluate: 7e-07	113	3	20	185		0.92	0.35	
contig18479:1:560:3 similar to Protein SCO2 homolog, mitochondrial (A6H784) Evaluate: 3e-51	111	2	13.5	185			0.34	
contig18463:388:801:1 similar to Motile sperm domain-containing protein 2 (Q8NHP6) Evaluate: 1e-11	64	2	21	138	0.48		0.44	
contig18335:1:1523:3 similar to Homogentisate 1,2-dioxygenase (Q9VKJ0) Evaluate: 4e-171	449	10 (9)	21.1	506	0.64	0.64	0.73	
contig18321:1:789:3 similar to Chymotrypsin-like elastase family member 2A (P08419) Evaluate: 3e-23	365	9 (6)	29.4	262		1.6	1.76	
contig18181:156:1403:3 similar to C-terminal-binding protein (O46036) Evaluate: 1e-165	96	2	4.3	416	0.55	0.15	0.37	
contig18178:1:889:3 similar to Aldose 1-epimerase (P05149) Evaluate: 2e-45	303	6 (5)	26.8	295	0.56	1.02	1.11	
contig18165:376:576:1 similar to Profilin-3 (Q21193) Evaluate: 3e-09	108	3 (2)	37.3	67	1.72			
contig18144:1:569:3 similar to CD63 antigen (Q9XSK2) Evaluate: 4e-15	77	2	11.2	188	0.48	0.5		
contig18027:84:1786:3 similar to Arylsulfatase J (Q8BM89) Evaluate: 2e-69	597	14 (12)	24.3	567		1.6	1.95	
contig18022:1:620:3 similar to Nucleosome assembly protein 1-like 1 (Q28EB4) Evaluate: 5e-20	217	6 (4)	18.4	206	2.04	1.02	0.76	
contig18018:83:1036:2 No distinct annotation Best-hit: RNA-binding motif protein, X-linked 2 (Q8R0F5) Evaluate: 3e-47	26	1	3.2	317		0.18		
contig18016:1:528:1 similar to ADP-ribosylation factor 1 (P61210) Evaluate: 2e-97	266	6 (5)	37.1	175	7.07	1.89		
contig17905:1:561:1 similar to Fumarylacetoacetase (A5PKH3) Evaluate: 6e-66	351	12 (7)	33.9	186	1.14	2.4	2.4	
contig17845:1:2472:1 similar to Neutral alpha-glucosidase C (Q8TET4) Evaluate: 0.0	741	20 (14)	28.9	823	0.41	0.96	0.56	
contig17822:1:826:2 No distinct annotation Best-hit: PDZ and LIM domain protein 3 (Q9PU47) Evaluate: 8e-09	329	9 (5)	29.2	274		2.06	1.93	
contig17821:1:1207:2 similar to Putative deoxyribonuclease TATDN2 (Q93075) Evaluate: 1e-38	72	2	6.7	401		0.15		
contig17805:111:1346:3 similar to Basigin (P17790) Evaluate: 2e-16	596	15 (11)	30.4	411	0.91	2.81	2.65	
contig17784:1:724:2 similar to Metaxin-2 (O75431) Evaluate: 3e-43	121	3	17.5	240	0.25	0.52	0.25	
contig17718:200:1417:2 similar to Minor histocompatibility antigen H13 (Q8TCT9) Evaluate: 9e-108	86	1	4.2	405	0.14	0.28	0.29	
contig17716:435:1397:3 similar to Carbonic anhydrase 2 (Q8UWA5) Evaluate: 5e-50	371	7	24.7	320	0.28	2.65	2.04	
contig17700:28:584:1 similar to Glucosidase 2 subunit beta (P14314) Evaluate: 2e-38	65	2	9.7	185		0.5		
contig17690:100:1038:1 similar to Hsp70-binding protein 1 (Q99P31) Evaluate: 4e-29	57	2	7.1	312	0.2			
contig17682:164:458:2 similar to Charged multivesicular body protein 1a (Q6PHF0) Evaluate: 5e-32	65	2	22.4	98		0.7		
contig17680:1:142:2 similar to GTPase KRas (P79800) Evaluate: 6e-15	99	2	51.1	47	2.38			
contig17678:1:887:3 similar to Mitochondrial carnitine/acylcarnitine carrier protein (Q9Z2Z6) Evaluate: 2e-107	154	4 (3)	14.6	294	0.34	0.82	0.48	
contig17669:1:705:3 similar to OCIA domain-containing protein 1 (Q9W1X9) Evaluate: 1e-24	43	1	9.8	234	0.26	0.26	1.09	
contig17641:106:1112:1 similar to Adenosine deaminase (B2T672) Evaluate: 7e-81	330	9 (6)	20.3	335	0.18	1.88	2.8	
contig17623:1:1091:1 similar to Pyruvate carboxylase 1 (O17732) Evaluate: 1e-91	136	3	9.1	363		0.7	0.61	
contig17622:1:709:3 similar to Polyadenylate-binding protein (P21187) Evaluate: 1e-99	562	12 (10)	55.7	235	5.11	8.59	7.63	
contig17587:183:1079:3 similar to 3-hydroxyanthranilate 3,4-dioxygenase (P46952) Evaluate: 5e-62	164	3	12.8	298		0.31	0.2	

## Supplementary

contig17574:138:1355:3 similar to Sorting and assembly machinery component 50 homolog B (Q5U3I0) Evalue: 1e-50	293	6	20.2	405	0.14	0.77	0.6
contig17569:830:1939:2 similar to Uncharacterized protein L728 (Q5UNY4) Evalue: 2e-66	244	5 (4)	13.6	369	0.17	0.36	0.59
contig17569:142:840:1 similar to Uncharacterized protein L728 (Q5UNY4) Evalue: 7e-36	201	4	18.5	232		0.71	0.78
contig17535:1:360:1 similar to Acyl carrier protein, mitochondrial (P52505) Evalue: 2e-26	59	2	16.8	119	0.56		
contig17530:48:1571:3 seems to belong to epsin family Best-hit: Epsin-2 (Q9Z1Z3) Evalue: 3e-80	107	3	7.3	507		0.19	
contig17520:89:1249:2 similar to Deoxyribonuclease tatD (P27859) Evalue: 2e-51	127	2	6.5	386		0.15	
contig17518:1:952:2 similar to Torsin-1A-interacting protein 2 (Q6P752) Evalue: 7e-05	151	3	12.3	316	0.3		
contig17509:1:1216:2 similar to RING finger protein 160 (Q6A009) Evalue: 5e-53	181	5	16.1	404		0.4	0.15
contig17495:1:1150:2 similar to Torsin-like protein (Q95NU5) Evalue: 1e-63	188	4 (3)	10.5	382	0.24	0.24	0.16
contig17486:1:696:1 similar to Transcriptional activator protein Pur-alpha (Q00577) Evalue: 2e-45	195	4	17.7	231		0.98	0.72
contig17480:2216:2605:3 similar to Profilin (P25843) Evalue: 2e-25	59	1	7.8	129	12.81	0.5	
contig17435:1:136:2 similar to Protein archease (Q2YDE7) Evalue: 1e-09	58	1	20	45	1.3		
contig17429:135:597:3 similar to Testis-expressed sequence 264 protein (Q9Y6I9) Evalue: 2e-11	251	4	32.5	154	1.79	1.41	1.19
contig17427:294:692:3 similar to Cadherin-related tumor suppressor (P33450) Evalue: 4e-04	62	2	9.1	132	0.5		
contig17413:1:1829:3 similar to Bifunctional purine biosynthesis protein PURH (Q35567) Evalue: 0.0	411	8	17.1	608	0.46		
contig17304:1:341:2 similar to Aldo-keto reductase family 1 member C13 (Q8VC28) Evalue: 2e-25	68	2	23	113		1.18	
contig17280:86:829:2 similar to Mannose-P-dolichol utilization defect 1 protein (Q9R0Q9) Evalue: 2e-51	35	1	3.2	248		0.24	0.26
contig17271:2440:2919:1 similar to UPF0451 protein C17orf61 homolog (A4K526) Evalue: 1e-16	39	1	13.8	159	0.38		
contig17260:1:1003:2 similar to Nodal modulator 1 (Q15155) Evalue: 1e-55	84	1	4.2	334		0.27	0.19
contig17224:48:716:3 similar to AP-1 complex subunit beta-1 (Q08DS7) Evalue: 2e-43	169	4 (3)	18.9	222	0.28	0.64	0.41
contig17202:24:1487:3 similar to ATP-dependent RNA helicase DDX19A (Q3ZBV2) Evalue: 3e-106	213	4	11.7	487	0.4	0.33	0.42
contig16973:1:1246:2 similar to Proliferation-associated protein 2G4 (P50580) Evalue: 4e-96	564	15 (11)	23.9	414	1.37	1.59	1.14
contig16967:136:588:1 similar to Mitochondrial fission 1 protein (Q9CQ92) Evalue: 5e-23	190	5 (4)	30	150	1.26		
contig16874:1:518:3 similar to Drebrin-like protein B (Q6GM14) Evalue: 1e-08	92	2	19.2	172		0.39	
contig16800:1:1125:1 similar to Actin-binding protein anillin (Q9V4P1) Evalue: 4e-08	134	2	8	374	0.16		
contig16796:1:1478:3 similar to Selenium-binding protein 1-A (Q52KZ7) Evalue: 2e-123	96	2	5.9	491	0.18	0.12	0.18
contig16680:1:1162:2 similar to Centromere protein J (Q9HC77) Evalue: 4e-10	35	1	2.6	386			0.14
contig16604:102:1247:3 similar to Drebrin-like protein (Q9UJU6) Evalue: 2e-40	118	2	6.6	381			0.16
contig16579:1:332:3 similar to Pre-mRNA branch site protein p14 (Q9Y3B4) Evalue: 5e-51	158	3 (2)	23.9	109	0.61		
contig16536:1:410:1 similar to Cullin-associated NEDD8-dissociated protein 1 (A7MBJ5) Evalue: 6e-17	59	2	12.5	136	0.44	0.49	
contig16460:100:1236:1 similar to Adenosylhomocysteinase B (O93477) Evalue: 4e-159	209	6 (4)	10.3	378		0.26	
contig16351:1:686:3 similar to Deoxyhypusine hydroxylase (Q66KT3) Evalue: 4e-56	131	2	10.1	227	0.28		0.28
contig16337:1:450:2 similar to Pyruvate carboxylase, mitochondrial (Q29RK2) Evalue: 9e-53	86	4 (2)	13.4	149		1.09	0.4
contig16244:1:1489:2 similar to Adenosylhomocysteinase (P10760) Evalue: 0.0	765	23 (12)	36	495	2.73	2.66	2.59
contig16234:30:495:3 similar to Disorganized muscle protein 1 (Q18066) Evalue: 1e-12	98	2	12.3	155		2.43	1.23
contig16141:83:745:2 similar to High choriolytic enzyme 1 (P31580) Evalue: 3e-25	250	5 (4)	30.3	221	1.31		
contig15999:1:517:2 similar to Pre-mRNA-processing factor 19 (Q9JMJ4) Evalue: 5e-35	109	2	20.5	171	0.39		
contig15825:1:1734:1 similar to Beta-hexosaminidase subunit alpha (Q0V8R6) Evalue: 3e-149	358	8 (7)	13.9	577	0.36	1.3	1.43
contig15711:416:748:2 similar to Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial (Q9CRB9) Evalue: 2e-10	84	2	20	110	0.58	0.84	1.49
contig15211:183:773:3 similar to Transducin-like enhancer protein 1 (O42469) Evalue: 2e-21	167	3	23.4	197	0.55		
contig15131:1:455:3 similar to Pyruvate carboxylase, mitochondrial (P11498) Evalue: 7e-40	72	2	15.2	151		0.65	0.65

## Supplementary

contig14556:130:966:1 similar to Cell division control protein 2 homolog (P23572) Evalue: 1e-91	155	3	12.2	279	0.34		
contig14545:1:529:2 similar to RNA-binding protein Nova-1 (Q2PFW9) Evalue: 2e-26	156	3	24.4	176		0.62	1.23
contig14497:1:864:3 similar to Sucrase-isomaltase, intestinal (P07768) Evalue: 2e-57	463	9 (6)	35.9	287		1.37	0.2
contig14480:1:676:2 similar to PGAP2-interacting protein (Q9H720) Evalue: 2e-72	71	1	5.3	225		0.26	0.39
contig14455:1:1118:3 similar to Solute carrier family 25 member 43 (Q5U3V7) Evalue: 2e-21	95	2	7	371	0.16	0.32	0.16
contig14290:36:851:3 similar to Ubiquilin-4 (Q9NRR5) Evalue: 8e-43	104	3 (2)	11.8	271			0.39
contig14045:1:508:2 similar to CCR4-NOT transcription complex subunit 1 (A0JP85) Evalue: 4e-17	208	4	26.8	168	0.92		
contig13787:1:2105:3 similar to Mucolipin-3 (Q8R4F0) Evalue: 3e-104	105	3	4.4	700	0.13		
contig13660:162:1127:3 similar to Glycogen debranching enzyme (P35574) Evalue: 3e-43	85	2	7.2	321		0.19	
contig13442:163:1686:1 similar to Synaptotagmin-1 (P41823) Evalue: 6e-124	167	2	5.7	507		0.12	
contig13310:1:464:1 similar to Glycogenin-2 (O15488) Evalue: 2e-38	45	1	5.2	154		0.6	
contig13209:362:1009:2 similar to Kin of IRRE-like protein 2 (Q6UWL6) Evalue: 3e-05	97	2	13.5	215	0.3		
contig13118:1:839:3 similar to AP-3 complex subunit delta-1 (O14617) Evalue: 6e-98	39	1	4.3	279			0.22
contig13117:73:1695:1 similar to EH domain-containing protein 1 (Q9H4M9) Evalue: 0.0	269	7 (6)	11.1	540	0.11	0.42	1.08
contig13096:1:1606:2 similar to Probable ATP-dependent RNA helicase DHX37 (Q8IY37) Evalue: 8e-85	59	2	3	535	0.12	0.11	
contig13094:290:1009:2 similar to Endoribonuclease Dicer (Q25BN1) Evalue: 4e-33	64	2	8.4	239	0.26		
contig13000:45:1155:3 similar to Soluble guanylate cyclase 88E (Q8INF0) Evalue: 1e-91	32	2 (1)	2.4	370	0.32	0.32	0.16
contig12605:133:1725:1 similar to Protein regulator of cytokinesis 1 (Q99K43) Evalue: 5e-21	34	2 (1)	1.1	530		0.15	0.2
contig12544:272:1150:2 similar to Homeobox protein orthopedia (Q7YTC2) Evalue: 2e-38	37	1	2.1	292		0.3	0.1
contig12239:1:752:3 No distinct annotation Best-hit: Nitrogen regulatory protein GLN3 (P18494) Evalue: 5e-12	30	1	2.8	249		0.24	0.24
contig12204:339:753:3 similar to UHRF1-binding protein 1-like (Q6NRZ1) Evalue: 6e-07	216	5 (4)	34.8	138	0.84	1.77	1.13
contig12091:1:1368:1 similar to Protein hu-li tai shao (Q02645) Evalue: 5e-69	234	5 (4)	12.1	455	0.21	0.34	0.46
contig12079:1:1127:2 similar to Nucleolar protein 58 (Q9Y2X3) Evalue: 7e-123	32	1	2.7	375	0.16		
contig12053:1:1528:2 similar to 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (Q0STD7) Evalue: 4e-135	806	22 (14)	39	508	0.33	1.75	1.54
contig11974:1:2093:3 similar to Nuclear cap-binding protein subunit 1 (C0H906) Evalue: 5e-121	163	3	4.2	696	0.13		
contig11915:1:694:2 similar to Roquin (Q5TC82) Evalue: 5e-39	27	1	3	231			0.24
contig11800:1:1381:2 similar to Putative maltase-glucoamylase-like protein FLJ16351 (Q6ZN80) Evalue: 6e-32	56	2	4.8	460		0.19	
contig11786:1:1513:2 similar to AP-2 complex subunit alpha-2 (Q0VCK5) Evalue: 9e-122	462	10	23.9	503	0.42	0.91	0.44
contig11741:111:1604:3 similar to Protein spinster homolog 1 (A8WGF7) Evalue: 2e-122	80	2	5.2	497	0.18		
contig11655:1:1386:1 similar to Mitochondrial Rho GTPase 1-A (Q6NVC5) Evalue: 1e-103	168	4	11.5	462	0.2	0.27	0.27
contig11628:942:2504:3 similar to Translocation protein SEC63 homolog (Q8VHE0) Evalue: 3e-35	85	2	3.3	520	0.11		
contig11628:226:942:1 similar to Translocation protein SEC63 homolog (Q8VHE0) Evalue: 1e-55	47	1	4.2	238	0.24		
contig11617:125:1984:2 similar to Leucine-rich repeat protein SHOC-2 (Q8AVI4) Evalue: 3e-176	62	2	3.4	619		0.15	
contig11611:137:1597:2 similar to Wings apart-like protein homolog (Q65Z40) Evalue: 4e-17	184	4	9.1	486	0.26		
contig11578:819:2354:3 similar to UPF0555 protein KIAA0776 homolog (Q4R367) Evalue: 2e-44	90	2	7.8	511	0.18	0.12	0.25
contig11536:402:2144:3 similar to NADP-dependent malic enzyme (P13697) Evalue: 0.0	901	16 (15)	51.6	580		1.08	0.41
contig11518:81:1472:3 similar to Protein F37C4.5 (O44400) Evalue: 2e-20	67	1	3.2	463			0.12
contig11427:1:1897:2 similar to Plastin-1 (Q3V0K9) Evalue: 6e-172	217	5	10	631		0.25	0.19
contig11311:232:1674:1 similar to Chaperone activity of bc1 complex-like, mitochondrial (Q5BJQ0) Evalue: 2e-150	215	4	13.5	480	0.12	0.27	0.6
contig11258:1:1283:3 similar to Adenosylhomocysteinase (P23526) Evalue: 0.0	146	4	9.4	427		0.29	0.71
contig11209:1:1451:3 similar to Fermitin family homolog 1 (Q5R8M5) Evalue: 2e-120	446	9 (7)	21.6	482		0.58	0.67

contig11166:2372:2832:3 similar to Protein fucU homolog (A2VDF0) Evalue: 3e-46	242	9 (5)	48	152	1.71			
contig11158:129:893:3 similar to Protein unc-112 (Q18685) Evalue: 1e-38	344	6	29.4	255	0.24	1.03	0.72	
contig11055:246:1975:3 similar to Sphingomyelin phosphodiesterase (P17405) Evalue: 2e-122	101	2	4.7	576	0.1			
contig11007:1:314:1 similar to Staphylococcal nuclease domain-containing protein 1 (Q7ZT42) Evalue: 2e-17	261	4	56.7	104	0.6	2.47	1.87	
contig11006:1:669:1 similar to Staphylococcal nuclease domain-containing protein 1 (Q7ZT42) Evalue: 1e-54	457	10 (7)	38.7	222	0.86	4.71	2.19	
contig10938:1:443:2 similar to Leucine-rich repeats and immunoglobulin-like domains protein 1 (Q96JA1) Evalue: 1e-08	100	3	17.7	147		0.73	0.73	
contig10913:1:706:3 similar to Mitotic checkpoint protein BUB3 (O43684) Evalue: 1e-68	116	3	15.4	234	0.54		0.42	
contig10895:82:3300:1 similar to Lysosomal alpha-glucosidase (Q9MYM4) Evalue: 0.0	752	17 (15)	18.9	1072		0.95	0.87	
contig10852:243:1415:3 similar to Uroporphyrinogen decarboxylase (Q9PTS2) Evalue: 2e-122	249	4	13.6	390		0.33	0.54	
contig10786:499:723:1 similar to Cullin-4A (Q13619) Evalue: 1e-09	59	2	24.3	74			0.38	
contig10782:1:663:3 similar to Vascular endothelial growth factor receptor 1 (P17948) Evalue: 3e-22	176	3	17.7	220		0.47	0.29	
contig10778:1:690:1 similar to Acyl carrier protein, mitochondrial (Q94519) Evalue: 2e-23	227	7 (5)	24.9	229	0.84			
contig10641:388:1797:1 similar to Solute carrier family 40 member 1 (Q9NP59) Evalue: 3e-64	32	1	2.6	469	0.24			
contig10640:590:2686:2 similar to Mannosyl-oligosaccharide glucosidase (O88941) Evalue: 1e-144	108	3	4.9	698	0.13			
contig10576:55:1821:1 similar to Protein Shroom3 (Q9QXN0) Evalue: 1e-16	29	1	1.4	588	0.05	0.1	0.05	
contig10522:217:1974:1 similar to Phosphatidylinositol-binding clathrin assembly protein LAP (Q9VI75) Evalue: 4e-128	188	4 (3)	7.7	585		0.32	0.1	
contig10467:284:2131:2 similar to Testis-expressed sequence 2 protein (Q8IWB9) Evalue: 6e-19	102	3	5.5	615		0.3	0.15	
contig10383:1:1176:1 similar to DNA replication licensing factor MCM5 (Q0V8B7) Evalue: 2e-160	89	2	7.1	392	0.16			
contig10253:1:1347:2 similar to Nodal modulator 1 (Q15155) Evalue: 3e-51	67	2	4.5	448			0.14	
contig10248:1:282:2 similar to Staphylococcal nuclease domain-containing protein 1 (Q7KZF4) Evalue: 2e-26	165	3	44.1	93	0.77	3.12	1.32	
contig10216:183:673:3 similar to Pyridoxine-5--phosphate oxidase (Q91XF0) Evalue: 6e-28	167	3	23.3	163		1.04	0.57	
contig10149:1:217:2 similar to Kynurenine--oxoglutarate transaminase 3 (Q6YP21) Evalue: 2e-11	93	2	39.4	71	1.42		1.84	
contig10073:201:2207:3 similar to Putative glycerophosphodiester phosphodiesterase 5 (Q9NPB8) Evalue: 5e-98	143	3	5.2	668		0.13	0.08	
contig10020:1:775:2 similar to Dystroglycan (Q62165) Evalue: 3e-09	94	2	10.1	257		0.25	0.24	
contig09995:1:599:2 similar to Polyadenylate-binding protein 2 (Q7KNF2) Evalue: 7e-40	190	3	20.1	199	0.66			
contig09979:1:389:1 similar to Phosphoacetylglucosamine mutase (O95394) Evalue: 1e-14	109	2	15.5	129		0.52	0.52	
contig09906:1:1312:2 similar to Calmodulin-1 (P62148) Evalue: 8e-83	97	2	6	436	0.14			
contig09773:216:1790:3 similar to Cubilin (O70244) Evalue: 2e-55	49	1	1.9	524		0.12		
contig09697:1:361:3 similar to Toll-like receptor 9 (Q512M3) Evalue: 6e-09	186	2	35.3	119		0.6	1.55	
contig09589:170:2278:2 similar to Exocyst complex component 3 (Q62825) Evalue: 1e-99	80	2	2.8	702		0.08		
contig09543:1:1091:3 similar to Uncharacterized protein C22orf9 homolog (Q801S4) Evalue: 1e-66	32	1	2.5	362		0.16		
contig09531:32:539:2 similar to Integrin beta-7 (P26010) Evalue: 3e-23	72	2	14.2	169			0.38	
contig09412:77:457:2 similar to Mitogen-activated protein-binding protein-interacting protein (B5FYY5) Evalue: 7e-46	163	2	22.2	126	0.56			
contig09345:73:483:1 similar to Probable multifunctional protein ADE2 (Q10457) Evalue: 1e-30	182	4	23.4	137	6.43	1.2	0.81	
contig09306:395:2317:2 similar to Propionyl-CoA carboxylase alpha chain, mitochondrial (Q612F5) Evalue: 0.0	522	13	28.6	640	0.14	0.96	0.71	
contig09304:158:472:2 similar to Aldose reductase-related protein 1 (P21300) Evalue: 2e-21	151	4	46.2	104		3.62	1.85	
contig09248:189:377:3 similar to Omega-amidase NIT2 (Q9NQR4) Evalue: 7e-15	47	1	17.5	63		1.04		
contig09151:222:1838:3 similar to Arylsulfatase B (P50429) Evalue: 3e-93	162	3	6.9	538		0.17	0.24	
contig08957:1:769:2 similar to Protein PRRC1 (Q96M27) Evalue: 2e-16	125	2	12.5	255		0.25	0.39	
contig08938:54:2429:3 similar to Mitofusin-2 (O95140) Evalue: 2e-157	104	2	3.4	791		0.07		
contig08881:59:1132:2 similar to Leucine-rich repeat-containing G-protein coupled receptor 6 (Q9HBX8) Evalue: 2e-17	243	4	14	357		0.37	0.17	

## Supplementary

contig08744:23:845:2 similar to Prominin-1 (O54990) Evalue: 4e-13	167	2	11.3	274		0.22		
contig08595:1:149:1 similar to Nucleolar protein 56 (O94514) Evalue: 2e-04	209	4	63.3	49	3.77	8.39	7.03	
contig08567:1:681:1 similar to Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (Q9R1D7) Evalue: 7e-39	104	2	11.9	227		0.28		
contig08460:1:768:1 similar to Lupus La protein homolog (P38656) Evalue: 6e-19	89	2	8.2	256		0.24	0.24	
contig08405:145:1326:1 similar to Arylacetamide deacetylase (Q0P5B7) Evalue: 4e-42	360	9 (8)	28.5	393		0.77	0.97	
contig08312:1:1199:3 similar to Integrin alpha ina-1 (Q03600) Evalue: 2e-30	64	2	6	398		0.37	0.22	
contig08274:1:2015:3 similar to Nuclear pore complex protein Nup88 (Q8CEC0) Evalue: 1e-13	60	2	3.4	670	0.13	0.09	0.09	
contig08270:1:796:2 similar to Integrin beta-PS (P11584) Evalue: 7e-57	165	3	16.6	265		0.37	0.23	
contig08238:193:2135:1 similar to Putative ATP-dependent RNA helicase an3 (P24346) Evalue: 2e-170	1536	45 (27)	52.2	647	2.81	5.13	4.2	
contig08228:1:594:1 similar to Single-stranded DNA-binding protein, mitochondrial (P54622) Evalue: 2e-28	178	5	28.4	197	0.84			
contig08224:1485:2588:3 similar to Secretory carrier-associated membrane protein 1 (P56603) Evalue: 8e-44	91	2	7.1	367	0.25			
contig08217:1:1689:1 similar to CCR4-NOT transcription complex subunit 1 (A1A5H6) Evalue: 3e-92	388	9	19.4	562	0.61			
contig08204:1:396:1 similar to Translocator protein (P30536) Evalue: 1e-25	55	1	7.6	131	0.46			
contig08150:364:982:1 similar to Cleavage and polyadenylation specificity factor subunit 5 (Q9CQF3) Evalue: 8e-72	117	3	18.4	206	0.48	0.48	0.48	
contig08149:1:734:2 similar to Nucleolin (P20397) Evalue: 2e-06	185	5 (4)	18.9	244	1.08	2.7	2.25	
contig08104:1:628:3 similar to Kynurenine--oxoglutarate transaminase 3 (Q71R19) Evalue: 1e-40	230	5	31.2	208	0.65	1.12	0.87	
contig08054:1:264:3 similar to Transforming growth factor beta-1-induced transcript 1 protein (Q2TCH4) Evalue: 5e-30	80	2	43.7	87		0.75	0.64	
contig07993:1:177:1 similar to Charged multivesicular body protein 2a (Q7ZW25) Evalue: 4e-04	177	3 (2)	32.8	58	1.6		1.6	
contig07966:116:1501:2 similar to Protein lunapark (Q9C0E8) Evalue: 9e-30	67	1	2.6	461	0.19	0.12	0.19	
contig07793:158:1669:2 similar to Glutamate decarboxylase-like protein 1 (Q80WP8) Evalue: 8e-119	121	3	6	503			0.18	
contig07722:1:629:3 similar to Basement membrane proteoglycan (Q06561) Evalue: 5e-32	69	2	14.9	208		0.46	0.77	
contig07665:1:1771:2 similar to Extracellular domains-containing protein CG31004 (Q0KH3) Evalue: 5e-63	223	5	13.2	589		0.84	0.62	
contig07583:24:941:3 similar to Lipoma-preferred partner homolog (Q5F464) Evalue: 8e-70	139	2	9.5	305		0.28	0.3	
contig07535:1:397:2 similar to U6 snRNA-associated Sm-like protein LSM5 (Q2HJH0) Evalue: 5e-34	32	1	6.9	131	0.46			
contig07521:1:1472:3 similar to Tudor domain-containing protein 1 (A9CPT4) Evalue: 6e-10	67	2	4.5	489		0.13		
contig07521:1:1472:3 similar to Tudor domain-containing protein 1 (A9CPT4) Evalue: 6e-10	779	21 (16)	40.9	489	2.64			
contig07499:1422:1734:2 similar to Charged multivesicular body protein 2b (Q6NXD2) Evalue: 4e-16	48	1	10.7	103	0.6			
contig07499:1:566:3 similar to Protein mago nashi homolog 2 (Q0VC92) Evalue: 1e-66	137	5 (3)	18.2	187	0.67			
contig07490:1:489:1 similar to UDP-glucose 4-epimerase (Q14376) Evalue: 1e-62	269	7 (6)	42	162	1.84	1.84	2.38	
contig07467:75:1523:3 similar to ATP-dependent DNA helicase recQ (P15043) Evalue: 5e-39	38	1	1.5	482	0.19	0.12	0.41	
contig07454:1:868:2 similar to Calcium-binding protein 39 (Q29R16) Evalue: 3e-99	49	1	3.1	288	0.64	0.2	0.42	
contig07386:1:1873:2 similar to Eukaryotic peptide chain release factor GTP-binding subunit ERF3A (P15170) Evalue: 2e-158	749	16 (14)	28.9	623	0.66	0.91	1.1	
contig07280:1:317:3 similar to Beta-ureidopropionase (Q5RBM6) Evalue: 2e-21	135	2	30.8	104	0.99			
contig07271:1:693:1 similar to Mitochondrial 2-oxoglutarate/malate carrier protein (Q02978) Evalue: 1e-83	191	3	18.7	230		0.46	1.4	
contig07264:1:215:1 similar to Transforming protein RhoA (P24406) Evalue: 1e-23	58	2	28.2	71			1.02	
contig07244:1:968:2 similar to AP-1 complex subunit beta-1 (Q10567) Evalue: 2e-159	635	14 (11)	44.7	322	0.85	2.76	1.49	
contig07229:1874:2555:2 similar to Prostaglandin-E(2) 9-reductase (P80508) Evalue: 1e-30	371	8 (7)	39.4	226		1.44	1.31	
contig07229:1874:2555:2 similar to Prostaglandin-E(2) 9-reductase (P80508) Evalue: 1e-30	342	9 (7)	25.2	226			0.16	
contig07167:60:1904:3 similar to Gephyrin (Q03555) Evalue: 9e-109	116	3	4.7	614			0.1	
contig07147:1:1064:3 similar to Ligatin (Q61211) Evalue: 2e-19	129	2	7.9	353		0.17		

## Supplementary

contig07141:1:202:1 similar to Astacin (P07584) Evalue: 3e-05	101	2 (1)	20.9	67	1.63	0.94	0.94
contig07114:261:717:3 similar to Acyl-protein thioesterase 1 (P97823) Evalue: 2e-37	137	4 (3)	34.2	152	0.66	1.2	0.96
contig07105:169:1788:1 similar to Fimbrin (P32599) Evalue: 1e-103	71	2	4.1	539		0.11	
contig06996:1:916:2 similar to Integrin beta-PS (P11584) Evalue: 7e-99	74	2	13.4	305		0.2	
contig06973:139:612:1 similar to RNA-binding protein 8A (B5DGI7) Evalue: 7e-35	110	3 (2)	15.3	157	0.41		
contig06904:92:1099:2 similar to DNA-directed RNA polymerases I and III subunit RPAC1 (Q32L22) Evalue: 9e-60	24	1	3	335	0.18		0.18
contig06875:89:901:2 similar to Uncharacterized protein DR_0705 (Q9RWG3) Evalue: 3e-39	166	5	18.9	270		0.87	0.76
contig06873:1:812:3 similar to UBX domain-containing protein 4 (Q3ZBU9) Evalue: 3e-15	92	2	13.4	269			0.23
contig06872:1:1070:3 similar to 1,5-anhydro-D-fructose reductase (P82125) Evalue: 2e-72	201	10 (3)	9.6	355	0.25	0.34	0.76
contig06853:1:932:2 similar to Acid trehalase-like protein 1 (A0JMP0) Evalue: 5e-35	87	2	5.8	310		0.2	
contig06850:1:1176:1 similar to Maltase-glucoamylase, intestinal (O43451) Evalue: 2e-53	221	6 (5)	14.6	391		0.58	0.61
contig06824:88:915:1 similar to Chymotrypsin A (P47796) Evalue: 1e-44	816	25 (13)	60	275		6.77	6.47
contig06789:431:1138:2 similar to Putative OPA3-like protein CG13603 (Q9VCG3) Evalue: 6e-36	70	2	17.9	235			0.27
contig06765:39:331:3 similar to ADP-ribosylation factor-like protein 8A (Q96BM9) Evalue: 1e-48	92	2	21.6	97	1.2	0.69	0.69
contig06754:1629:2000:1 similar to Brain protein 44-like protein (P63031) Evalue: 4e-37	38	1	10.6	123	0.48		
contig06540:1:183:1 similar to Profilin-A (P22271) Evalue: 2e-11	150	4 (3)	75	60	5.12		
contig06460:96:881:3 similar to Kynurenine formamidase (Q2S2F5) Evalue: 2e-14	218	5 (4)	20.7	261	1.3	0.83	0.54
contig06408:1:315:3 similar to Phosphomannomutase 2 (Q86B09) Evalue: 7e-12	125	2	17.3	104		0.99	
contig06233:1:1734:1 similar to NMDA receptor-regulated 1-like protein (Q9DBB4) Evalue: 5e-106	260	7	14.9	577	0.15	0.39	0.21
contig06194:153:632:3 similar to Trafficking protein particle complex subunit 2 (Q9CQP2) Evalue: 2e-29	143	3	20.1	159	0.64		
contig06141:71:706:2 similar to DNA-directed RNA polymerases I, II, and III subunit RPABC1 (Q5R587) Evalue: 9e-90	218	6 (5)	31.3	211	0.88		
contig06054:375:617:3 similar to U6 snRNA-associated Sm-like protein LSm6 (P62312) Evalue: 1e-31	78	2	23.8	80	0.94		
contig06033:1:642:2 similar to Fasciclin-2 (P22648) Evalue: 6e-17	104	2	10.3	213	0.29		
contig05979:197:1027:2 similar to 4-hydroxyphenylpyruvate dioxygenase (Q5BKL0) Evalue: 2e-108	639	17 (9)	43	277	3.15	3.89	4.01
contig05948:250:1284:1 similar to Purine nucleoside phosphorylase (P00491) Evalue: 2e-90	123	3	11.6	344		0.47	0.58
contig05823:1:1097:3 similar to Proactivator polypeptide (P26779) Evalue: 7e-24	123	2	7.9	365	0.94	0.32	0.24
contig05804:90:1847:3 similar to Polypyrimidine tract-binding protein 2 (Q9UKA9) Evalue: 4e-143	391	8 (7)	15.2	585	0.59		
contig05791:115:1557:1 similar to FAS-associated factor 2 (Q96CS3) Evalue: 2e-69	165	2	6.2	480			0.12
contig05682:1:2558:3 similar to Tumor suppressor p53-binding protein 1 (Q12888) Evalue: 6e-22	187	4	5.5	851	0.34	0.18	0.19
contig05505:421:628:2 similar to UV excision repair protein RAD23 homolog A (P54725) Evalue: 4e-16	111	4 (2)	29.4	68	0.96	3.74	1.18
contig05482:1:399:3 similar to Extracellular domains-containing protein CG31004 (Q0KH3) Evalue: 8e-29	89	2	23.5	132		0.7	
contig05470:1:464:3 similar to Fructose-bisphosphate aldolase A (P05065) Evalue: 6e-54	528	17 (10)	68	153	3.69	16.75	14.34
contig05418:1:564:1 similar to GTP-binding nuclear protein GSP2/CNR2 (P32836) Evalue: 1e-79	80	2	12.2	188			0.34
contig05368:1:772:1 similar to Dihydropyrimidinase (Q63150) Evalue: 5e-95	252	5 (4)	22.6	257		0.94	1.15
contig05344:257:697:2 similar to UPF0195 protein CG30152 (Q9V968) Evalue: 2e-35	29	1	8.2	146	0.4		0.6
contig05343:1:367:2 similar to PRA1 family protein 2 (Q9JIG8) Evalue: 1e-08	40	1	5.8	121		0.5	0.5
contig05263:1:701:3 similar to Phosphatidylinositol transfer protein alpha isoform (P48738) Evalue: 6e-71	383	10 (9)	44.4	232	0.77	2.56	0.98
contig05218:1:1060:2 similar to Protein TTE1956 (Q8R8N9) Evalue: 3e-13	320	5	21.3	352	0.26	0.68	0.36
contig05199:158:1908:2 similar to Protein LSM14 homolog B (Q9BX40) Evalue: 1e-25	330	6 (5)	12	583	0.1	0.44	0.5
contig05176:15:484:3 similar to Uncharacterized protein ZC395.10 (Q23280) Evalue: 4e-13	66	2	11.5	156			0.41
contig05163:20:1228:2 similar to ATP-dependent RNA helicase fal1 (Q2UAK1) Evalue: 0.0	54	1	3.2	402	1.4	0.21	0.3

## Supplementary

contig05105:1:1266:1 similar to UPF0160 protein MYG1, mitochondrial (Q9JK81) Evalue: 4e-92	403	10	29.2	421	0.49	0.95	0.49
contig05104:1:388:2 similar to Polyadenylate-binding protein 1 (P29341) Evalue: 4e-13	27	1	4.7	128		0.5	0.82
contig05102:1:1215:1 similar to Sulfite oxidase (Q9S850) Evalue: 2e-81	414	8	27.7	404		0.75	0.75
contig05069:1:473:3 similar to Muscle LIM protein Mlp84B (Q24400) Evalue: 6e-28	87	5 (2)	16	156	1.09		
contig05012:1:318:1 similar to 4-hydroxyphenylpyruvate dioxygenase (P32754) Evalue: 2e-40	266	7 (4)	43.8	105	2.2	3.55	2.2
contig04923:1:653:3 similar to AP-3 complex subunit sigma-1 (Q2YDH6) Evalue: 4e-74	44	1	5.1	216	0.26		
contig04912:1:1264:2 similar to NSFL1 cofactor p47 (Q5ZK10) Evalue: 9e-62	84	3	7.9	420	0.23	0.23	
contig04894:99:1655:3 similar to Histone deacetylase 1 (Q5RAG0) Evalue: 0.0	146	3	8.5	518	0.63	0.18	0.18
contig04838:1:1178:3 similar to WD repeat-containing protein 36 (Q8NI36) Evalue: 2e-42	31	1	2	391	0.16		
contig04805:1:1752:1 similar to Protein misato homolog 1 (Q9BUK6) Evalue: 1e-73	108	2	3.8	583	0.15		
contig04791:1:1581:1 No distinct annotation Best-hit: PH and SEC7 domain-containing protein 3 (Q2PFD7) Evalue: 3e-40	78	2	4.8	526			0.11
contig04724:221:1534:2 similar to Alpha-N-acetylgalactosaminidase (Q90744) Evalue: 1e-120	372	9 (7)	19.9	437	0.14	0.96	1.29
contig04671:1:825:1 similar to Neural cell adhesion molecule 1 (P13596) Evalue: 2e-11	182	4	20.8	274	0.73		
contig04654:70:2148:1 similar to Rap1 GTPase-GDP dissociation stimulator 1-B (Q5PPZ9) Evalue: 2e-27	82	1	1.9	692	0.09	0.08	
contig04650:16:1878:1 similar to Protein PTCD3 homolog, mitochondrial (A1Z9A8) Evalue: 4e-82	167	4	7.7	620	0.09	0.2	0.09
contig04639:207:559:3 similar to Cellular retinoic acid-binding protein 1 (P40220) Evalue: 7e-11	213	5 (4)	51.3	117	1.43		
contig04560:1:333:1 similar to Phosphatidylethanolamine-binding protein 1 (P70296) Evalue: 1e-15	220	5	35.5	110		3.74	0.91
contig04558:1:896:3 similar to Pancreatic alpha-amylase (P00689) Evalue: 8e-102	216	4	16.4	298		0.46	0.21
contig04476:1:678:1 similar to Prefoldin subunit 5 (Q5RAY0) Evalue: 2e-13	293	5 (4)	17.8	225	1.04		
contig04448:197:1144:2 similar to Aldo-keto reductase family 1 member C23-like protein (Q6W8P9) Evalue: 1e-56	410	8	30.2	315		1.32	0.55
contig04415:1:828:1 similar to Protein KTI12 homolog (Q0P457) Evalue: 1e-55	68	2	6.9	275		0.22	0.35
contig04406:76:936:1 similar to Beta carbonic anhydrase 1 (Q22460) Evalue: 3e-53	233	8 (6)	23.8	286	0.47	1.15	0.78
contig04381:209:601:2 similar to GTPase NRas (Q5F352) Evalue: 3e-57	181	4	37.4	131	1.28		
contig04329:85:642:1 similar to Protein jagunal (Q297K8) Evalue: 7e-26	48	1	6.5	185	0.45		
contig04318:1:485:3 similar to Protein RER1 (Q5ZHM5) Evalue: 2e-55	89	1	10.6	160	0.54		
contig04290:260:1024:2 similar to DNA-directed RNA polymerases I, II, and III subunit RPABC3 (P52434) Evalue: 8e-52	116	3 (2)	9.1	254	0.24		
contig04236:28:1196:1 similar to Solute carrier family 12 member 5 (Q9H2X9) Evalue: 5e-68	43	2 (1)	1.5	389	0.21		
contig04219:1:362:2 similar to Band 7 protein CG42540 (Q9VZA4) Evalue: 5e-35	48	1	5.8	120		0.52	1.11
contig04202:1:563:3 similar to AP-2 complex subunit mu (P84091) Evalue: 6e-99	78	2	14.5	186	0.33		0.78
contig04086:1:987:2 similar to Putative deoxyribose-phosphate aldolase (Q3T0V9) Evalue: 2e-73	162	3	13.4	328	0.3		
contig03974:1:525:3 similar to Tolloid-like protein 2 (Q9WVM6) Evalue: 9e-14	256	4	28.2	174		1.1	1.26
contig03973:162:1427:3 similar to Histone-binding protein RBBP4 (Q6P3H7) Evalue: 0.0	163	5 (4)	9.5	421	1.03	0.39	0.14
contig03956:1:1116:1 similar to Sodium-driven chloride bicarbonate exchanger (Q6U841) Evalue: 8e-105	130	4 (3)	10.2	371		0.51	0.32
contig03937:165:1900:3 similar to Centromere/kinetochore protein zw10 homolog (Q54692) Evalue: 1e-09	35	1	1.2	578	0.1		
contig03929:285:1313:3 similar to Twinfilin-1 (Q51082) Evalue: 2e-97	119	3	10.2	342		0.46	0.36
contig03920:1:710:3 similar to Ras suppressor protein 1 (Q5E9C0) Evalue: 6e-82	289	7 (4)	31.5	235	0.6	0.92	0.6
contig03901:1:342:3 similar to Cytoskeleton-associated protein 5 (Q14008) Evalue: 2e-20	54	2	22.1	113	0.84		
contig03897:1:396:1 similar to Histidine triad nucleotide-binding protein 2 (Q9D0S9) Evalue: 6e-38	110	4 (3)	30.5	131	1.11		
contig03896:1:1169:1 similar to Beta-centractin (Q8R5C5) Evalue: 4e-142	256	4	17.7	389	0.34	0.34	0.24
contig03888:1540:2081:3 similar to UPF0553 protein C9orf64 (Q5T6V5) Evalue: 4e-48	63	2	13.4	179	0.55	0.34	0.34
contig03850:179:2113:2 similar to Nuclear protein localization protein 4 homolog (Q8TAT6) Evalue: 0.0	171	3	7	644	0.14	0.14	0.3

## Supplementary

contig03819:1:635:3 similar to 1,5-anhydro-D-fructose reductase (Q9DCT1) Evalue: 6e-37	313	5	35.2	210		1.19	0.9
contig03806:1:678:1 similar to Ophanin (Q7ZT98) Evalue: 5e-23	154	10 (4)	23.6	225	1.68		0.27
contig03798:1:1418:3 similar to Tetratricopeptide repeat protein 27 (Q17QZ7) Evalue: 3e-85	135	2	7.6	471		0.12	
contig03757:1:953:3 similar to Protoporphyrinogen oxidase (P51175) Evalue: 2e-40	148	3	15.1	317		0.31	
contig03695:118:2208:1 similar to Acylamino-acid-releasing enzyme (Q8R146) Evalue: 8e-131	582	15 (14)	20.5	697	0.33	0.84	0.21
contig03675:297:1100:3 similar to Chitosanase (O07921) Evalue: 3e-33	206	5 (4)	18.7	267		0.52	0.37
contig03620:28:725:1 similar to Glucan endo-1,3-beta-glucosidase A1 (P23903) Evalue: 2e-16	84	2	9.5	232		0.27	
contig03576:123:971:3 similar to 1,25-dihydroxyvitamin D(3) 24-hydroxylase, mitochondrial (Q07973) Evalue: 3e-18	291	7 (5)	28.7	282		0.83	0.73
contig03546:104:1024:2 similar to WAP four-disulfide core domain protein 3 (Q8IUB2) Evalue: 4e-11	185	5	19.5	307	0.18	0.66	1.06
contig03490:1845:2819:1 similar to Integrin beta pat-3 (Q27874) Evalue: 1e-63	137	3	9.3	324		0.29	
contig03461:93:575:3 similar to Cysteine-rich motor neuron 1 protein (Q7T3Q2) Evalue: 4e-09	84	1	9.4	160	2.19		
contig03449:108:409:3 similar to ADP-ribosylation factor-like protein 6-interacting protein 1 (Q5R454) Evalue: 5e-05	87	1	19	100	1.02		
contig03443:1:1247:3 similar to mRNA export factor (Q7ZWF0) Evalue: 1e-124	304	5 (4)	15.7	414	0.4	0.31	0.5
contig03439:354:885:3 similar to CDK5 regulatory subunit-associated protein 3 (Q99LM2) Evalue: 1e-28	100	4 (2)	13	177		0.34	0.55
contig03434:1:1420:2 similar to 2-oxoglutarate and iron-dependent oxygenase domain-containing protein 1 (Q6DE73) Evalue: 1e-39	145	3	6.6	472		0.19	
contig03419:1:945:1 similar to VIP36-like protein (P59481) Evalue: 2e-73	148	3	15.6	315	0.19		
contig03411:1:284:3 similar to 1,4-alpha-glucan-branching enzyme (Q6FJV0) Evalue: 3e-25	187	3	42.6	94		1.29	1.29
contig03407:1:1094:3 No distinct annotation Best-hit: SET and MYND domain-containing protein 4 (Q8IYR2) Evalue: 3e-08	146	3	9.6	363	0.16		
contig03356:150:701:3 similar to Vitamin K epoxide reductase complex subunit 1-like protein 1 (Q6TEK5) Evalue: 5e-22	241	10 (5)	41	183	2.27		
contig03356:150:701:3 similar to Vitamin K epoxide reductase complex subunit 1-like protein 1 (Q6TEK5) Evalue: 5e-22	31	1	7.1	183			0.32
contig03353:100:1653:1 similar to Protein ERGIC-53 (Q9D0F3) Evalue: 3e-98	529	11 (10)	23.2	517	1.53	2.45	2.53
contig03342:1:1530:1 similar to Protein F37C4.5 (O44400) Evalue: 8e-44	676	13	30.5	509		1.47	1.38
contig03338:155:478:2 similar to OV-16 antigen (P31729) Evalue: 1e-10	26	1	9.3	108		0.58	
contig03333:1:274:2 similar to Mitotic checkpoint protein BUB3 (Q9WVA3) Evalue: 4e-12	78	2	17.8	90	1.13		
contig03277:146:1405:2 similar to Polyadenylate-binding protein-interacting protein 1 (Q9H074) Evalue: 2e-05	73	2 (1)	2.4	420	0.23	0.14	
contig03121:176:1441:2 similar to Mitochondrial inner membrane protein OXA1L (Q8BGA9) Evalue: 1e-65	29	1	2.4	421	0.14	0.14	0.07
contig03116:1:853:1 No distinct annotation Best-hit: Peflin (Q6DC93) Evalue: 3e-44	207	7 (4)	18.7	284		0.87	0.87
contig03074:175:2363:1 similar to Protein OS-9 (Q13438) Evalue: 1e-26	71	2	3	729	0.17	0.04	0.08
contig03067:284:1441:2 similar to PDZ and LIM domain protein 1 (O00151) Evalue: 3e-32	422	8 (6)	31.2	385	0.17	1.63	1.55
contig02987:1:559:2 similar to Replication protein A 14 kDa subunit (P35244) Evalue: 4e-09	81	3 (2)	17.3	185	0.93		
contig02971:1:970:2 similar to F-actin-capping protein subunit alpha (Q9W2N0) Evalue: 3e-99	266	8 (6)	22	322	0.5	0.67	0.67
contig02968:134:802:2 similar to Uncharacterized protein C6orf203 homolog (Q9CQF4) Evalue: 5e-06	284	5	29.7	222	0.83		
contig02913:137:517:2 similar to Transcription initiation factor IIA subunit 2 (P52656) Evalue: 3e-34	129	4 (3)	25.4	126	1.15		
contig02862:57:317:3 similar to BoIA-like protein 2 (Q8BGS2) Evalue: 1e-12	60	1	13.8	87	0.72		
contig02850:93:689:3 similar to Protein wos2 (Q11118) Evalue: 7e-22	201	6 (5)	22.2	198	2.48	2.43	2.13
contig02798:1:1462:2 similar to Probable cysteine desulfurase, mitochondrial (Q9VKD3) Evalue: 0.0	402	9 (8)	23	486	0.43	0.71	0.71
contig02791:294:1076:3 similar to Sorting nexin-18 (Q91ZR2) Evalue: 2e-47	173	3	20.4	260			0.5
contig02692:53:1738:2 similar to Basement membrane proteoglycan (Q06561) Evalue: 6e-05	1074	38 (16)	38.7	561	0.54	2.98	2.27
contig02679:141:2189:3 similar to Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial (Q99MR8) Evalue: 0.0	573	9	20.2	682	0.29	0.68	0.78
contig02660:89:739:2 similar to ADP-ribosylation factor 1 (P61210) Evalue: 5e-97	333	10 (7)	43.1	216	1.44		0.89



## Supplementary

contig02518:1:1076:3 similar to Protein amalgam (P15364) Evalue: 3e-13	123	3	10.1	357	0.17	0.27	0.17
contig02456:1:946:2 similar to Fruit bromelain (O23791) Evalue: 6e-24	65	3 (2)	4.5	314		0.2	
contig02439:1:958:2 similar to AFG3-like protein 2 (Q2KJ17) Evalue: 4e-133	114	2	7.9	318	0.42	0.28	0.55
contig02399:1:628:2 similar to Nuclear migration protein nudC (Q5ZIN1) Evalue: 3e-72	191	5	28.8	208	0.91	1.05	
contig02350:217:493:1 similar to PDZ and LIM domain protein 7 (Q679P3) Evalue: 2e-09	77	2	20.7	92			0.8
contig02295:1:333:2 similar to DNA-directed RNA polymerase II subunit RPB7 (P62489) Evalue: 2e-50	97	2	20.7	111		0.63	
contig02291:2219:2885:2 similar to Uncharacterized protein C6orf136 homolog (Q5TM64) Evalue: 2e-21	53	1	5	221		0.26	
contig02224:137:2384:2 similar to Lysosomal alpha-mannosidase (O09159) Evalue: 0.0	933	16 (15)	25.6	749	1.1	1.01	0.97
contig02202:133:651:1 similar to Putative defense protein (Q008X1) Evalue: 2e-10	217	7 (5)	31.4	172	2.34		
contig02192:158:652:2 similar to Sorting nexin-12 (Q9UMY4) Evalue: 7e-67	119	4 (3)	16.5	164	1.55	0.63	0.92
contig02175:85:681:1 similar to COMM domain-containing protein 4 (Q9CQ02) Evalue: 5e-30	30	1	4	198		0.3	
contig02168:1:786:1 similar to AP-2 complex subunit sigma (Q17QC5) Evalue: 3e-64	65	3 (2)	5.7	261	0.22		
contig02159:1:486:1 similar to Death-associated protein 1 (P51397) Evalue: 5e-05	57	1	7.5	161	0.38		0.41
contig02156:203:1449:2 similar to LIM and senescent cell antigen-like-containing domain protein 1 (Q99JW4) Evalue: 4e-117	238	6 (5)	14	415		0.59	0.35
contig02128:99:437:3 similar to Stefin-C (P35478) Evalue: 5e-15	209	6 (5)	51.8	112	3.26		
contig02123:143:1432:2 similar to Monomeric sarcosine oxidase (P23342) Evalue: 2e-50	288	8	24	429		0.76	0.39
contig02106:194:739:2 similar to Angiotensin-converting enzyme 2 (Q24K15) Evalue: 1e-15	30	1	6	182	0.32	0.48	
contig02083:1:416:1 similar to Carbonyl reductase [NADPH] 1 (Q28960) Evalue: 1e-20	89	2	12.3	138		0.49	0.49
contig02037:183:689:3 similar to Calcium-regulated heat stable protein 1 (Q9WU49) Evalue: 1e-29	59	2	10.7	168			0.39
contig02000:91:1617:1 similar to Sorting nexin-2 (O60749) Evalue: 1e-128	251	5	12.4	508	0.32	0.32	0.45
contig01996:41:293:2 similar to Stromal cell-derived factor 2 (Q3SZ45) Evalue: 2e-14	68	1	16.7	84	0.74	0.74	
contig01973:101:1333:2 similar to Lissencephaly-1 homolog B (Q803D2) Evalue: 8e-158	78	2	4.6	410	0.21	0.21	0.21
contig01877:1:296:3 similar to Dixin (Q155Q3) Evalue: 3e-08	26	1	8.2	97		2.96	
contig01875:1:640:1 similar to Histone deacetylase 10 (Q969S8) Evalue: 7e-56	97	3	18.8	213		0.46	
contig01826:1:238:2 similar to Mitochondrial glutamate carrier 1 (Q9D6M3) Evalue: 3e-12	58	1	15.4	78		1.6	1.05
contig01815:1:467:2 similar to Fasciclin-2 (P34082) Evalue: 1e-13	159	4	27.7	155	1.7		
contig01808:1:1057:2 similar to Carbonyl reductase [NADPH] 3 (O75828) Evalue: 1e-56	158	4	10.3	351		0.38	
contig01800:1:1033:2 similar to Glucosidase 2 subunit beta (O08795) Evalue: 6e-36	202	4 (3)	12.2	343	0.46	0.54	1
contig01764:1:419:3 similar to BET1-like protein (Q68EL3) Evalue: 1e-12	81	2	15.9	138	0.47		
contig01757:1:876:1 similar to Adenylyl cyclase-associated protein 1 (Q01518) Evalue: 1e-58	471	9	44.3	291	1.17	2.61	3.01
contig01739:211:885:1 similar to Ganglioside GM2 activator (Q60648) Evalue: 5e-32	46	1	6.7	224	0.72	0.26	
contig01724:145:861:1 similar to Dihydropteridine reductase (P11348) Evalue: 2e-62	498	16 (8)	47.1	238	2.7	3.11	1.84
contig01682:160:1764:1 similar to Protein prune homolog (Q86TP1) Evalue: 9e-49	380	6	16.1	534		0.38	0.24
contig01638:1:503:3 similar to Testis-expressed sequence 2 protein (Q8IWB9) Evalue: 2e-30	83	1	7.8	166	0.34		
contig01633:343:1557:1 similar to Cleavage stimulation factor 50 kDa subunit (Q05048) Evalue: 8e-90	38	1	3.2	404	0.14		
contig01616:104:1765:2 similar to Uncharacterized protein F07F6.4 (Q09531) Evalue: 7e-84	221	4	9	553	0.49	0.38	0.48
contig01608:13:996:1 similar to Abhydrolase domain-containing protein 11 (Q8NFV4) Evalue: 1e-46	130	3	13.1	327		0.3	0.54
contig01507:1:1108:2 similar to Carbonyl reductase [NADPH] 1 (Q28960) Evalue: 5e-56	87	3 (2)	3.8	368			0.17
contig01488:2063:3062:2 similar to Alpha-soluble NSF attachment protein (Q9DB05) Evalue: 7e-79	523	11 (8)	33.1	332	0.93	1.32	1.32
contig01483:137:1046:2 similar to Integral membrane protein 2B (Q5XIE8) Evalue: 9e-15	288	6	23.4	303	2.32		
contig01481:66:851:3 similar to DCN1-like protein 1 (Q96GG9) Evalue: 2e-64	243	7 (6)	25.7	261	0.23	0.86	0.67

## Supplementary

contig01454:273:1126:3 similar to ADP,ATP carrier protein 1 (Q27238) Evalue: 4e-103	264	7 (6)	20.8	284	1.76	3.5	3.63
contig01371:1:1262:2 similar to Interferon-related developmental regulator 1 (O00458) Evalue: 9e-40	73	2	4	420	0.31	0.15	0.23
contig01339:1433:2092:2 similar to Trafficking protein particle complex subunit 4 (Q9ES56) Evalue: 8e-67	187	2	15.1	219	0.29	0.29	
contig01319:101:1045:2 similar to Cartilage-associated protein (O75718) Evalue: 2e-23	94	2	6	315	0.18		
contig01292:1:1019:3 similar to Translocating chain-associated membrane protein 1-like 1 (Q6DED0) Evalue: 3e-64	126	4 (3)	10.6	339	0.25	1.23	1.58
contig01286:84:389:3 similar to Pre-mRNA-processing factor 39 (Q1Jpz7) Evalue: 2e-23	57	2	14.9	101		0.65	
contig01245:1:1384:2 similar to Cullin-associated NEDD8-dissociated protein 1 (Q6ZQ38) Evalue: 8e-105	670	15 (13)	30.7	460	1.01	1.61	1.01
contig01161:1:726:1 similar to Insulin-degrading enzyme (Q9JHR7) Evalue: 3e-58	227	4	23.6	242	0.41	0.41	
contig01128:1:1123:2 similar to Probable imidazolonepropionase (Q96NU7) Evalue: 8e-124	132	3	5.9	373		0.26	0.26
contig01126:186:312:3 similar to Polyadenylate-binding protein 1-B (Q6IP09) Evalue: 6e-15	75	1	26.2	42		1.62	
contig01073:1:1280:3 similar to L-allo-threonine aldolase (O07051) Evalue: 3e-75	147	3	11.3	425	0.15		0.23
contig01050:1:485:2 similar to Epididymal secretory protein E1 (Q9Z0J0) Evalue: 7e-08	77	2	5.6	161	1.47	0.79	0.38
contig01025:150:1202:3 similar to UPF0661 TPR repeat-containing protein C16D10.01c (Q1MTN8) Evalue: 1e-14	103	2	6.3	351			0.17
contig00978:106:1098:1 similar to Prolyl 4-hydroxylase subunit alpha-2 (Q20065) Evalue: 5e-38	173	3	12.4	331			0.28
contig00973:1:622:2 similar to Glycine cleavage system H protein, mitochondrial (Q9U616) Evalue: 1e-39	108	3	19.9	206	0.5		
contig00939:1:717:1 similar to Dihydropyrimidinase-related protein 3 (Q6GL72) Evalue: 1e-59	110	3	15.5	238		0.56	0.61
contig00840:83:622:2 similar to Ribonuclease UK114 (Q3T114) Evalue: 1e-41	127	5 (3)	22.9	179	0.92		
contig00803:1:631:2 similar to CDGSH iron sulfur domain-containing protein 3, mitochondrial (P0C7P0) Evalue: 1e-10	147	3	15.8	209	0.47		
contig00723:77:1030:2 similar to Protein SEC13 homolog (P55735) Evalue: 1e-67	221	4	14.8	317	0.85	0.42	0.19
contig00706:60:1345:3 similar to UPF0027 protein C22orf28 homolog (A9CB42) Evalue: 0.0	409	9	25.2	428	0.83		0.83
contig00649:1:686:3 similar to OV-16 antigen (P31729) Evalue: 4e-53	75	2	13.7	227	0.27		
contig00621:129:457:3 similar to ORM1-like protein 1 (Q29RQ9) Evalue: 4e-24	105	3	27.5	109	1.02		
contig00593:1:740:3 similar to Glucan endo-1,3-beta-glucosidase A1 (P23903) Evalue: 8e-25	73	2	14.2	246		0.24	
contig00589:1:683:1 similar to Nuclear localization sequence-binding protein (P27476) Evalue: 2e-12	148	2	15	227			0.29
contig00522:1:1380:1 similar to Spartin (Q8N0X7) Evalue: 3e-37	95	2	5.2	460			0.13
contig00499:96:428:3 similar to Hemiasterlin resistant protein 1 (Q09254) Evalue: 5e-13	41	1	10.9	110	0.56		
contig00389:54:533:3  conserved hypothetical protein [Ixodes scapularis] (XP_002411729.1) Evalue: 5e-15	31	1	6.9	159	1.44	0.54	2.49
contig06415:167:832:2  PREDICTED: nidogen 2 (osteonidogen) [Danio rerio] (XP_001919796.1) Evalue: 5e-07	226	6 (5)	23.5	221		0.75	0.57
contig18200:1:1065:2  unnamed protein product [Microcystis aeruginosa PCC 7806] (GI_159026529) Evalue: 7e-53	564	13 (11)	36.2	354		1.39	1.21
contig06067:1:2629:2  hypothetical protein TcasGA2_TC006727 [Tribolium castaneum] (GI_270012570) Evalue: 5e-19	75	1	2.1	875		0.15	0.06
contig07866:20:787:2  unnamed protein product [Tetraodon nigroviridis] (GI_47228124) Evalue: 1e-05	201	8 (5)	27.8	255		0.73	1.05
contig09662:91:480:1  hypothetical protein BRAFLDRAFT_78733 [Branchiostoma floridae] (XP_002612639.1) Evalue: 3e-11	132	3	22.3	130		0.85	1.36
contig10061:119:751:2  sarcoplasmic calcium-binding proteins I, III, and IV, putative [Ixodes scapularis] (XP_002434211.1) Evalue: 3e-10	133	2	11.9	210		0.13	0.28
contig07519:303:1775:3  AGAP003012-PA [Anopheles gambiae str (XP_311867.3) Evalue: 4e-40	90	2	4.5	490		0.12	
contig17102:266:784:2  Ganglioside GM2 activator precursor [Entamoeba dispar SAW760] (XP_001738333.1) Evalue: 2e-06	62	2 (1)	18	172		0.36	
contig01287:1:303:1  AGAP009865-PA [Anopheles gambiae str (XP_318983.3) Evalue: 5e-06	39	1	10	100		0.6	
contig02233:1:936:2  G113726 [Drosophila mojavensis] (XP_002002242.1) Evalue: 8e-14	28	1	1.9	311		0.27	
contig05501:40:1212:1  hypothetical protein BRAFLDRAFT_95854 [Branchiostoma floridae] (XP_002598738.1) Evalue: 2e-05	77	2	11.5	390		0.16	

## Supplementary

contig11422:1:587:3  G118881 [Drosophila mojavensis] (XP_002005794.1) Evalue: 6e-15	46	1	6.2	195	0.3		0.3
contig02716:1:796:2  novel protein [Danio rerio] (GI_148725447) Evalue: 6e-06	156	4	14.8	264		0.53	
contig02855:103:384:1  hypothetical protein AaeL_AAEL007490 [Aedes aegypti] (XP_001652797.1) Evalue: 4e-05	99	2	22.6	93		2.2	
contig00205:137:462:2  hypothetical protein Bm1_46440 [Brugia malayi] (XP_001900750.1) Evalue: 1e-10	165	3	38	108		1.1	
contig01342:1:461:3  PREDICTED: similar to Prominin 1 precursor (Prominin-like protein 1) (Antigen AC133) (CD133 antigen) isoform 1 [Canis familiaris] (XP_545934.2) Evalue: 5e-05	58	2	14.5	152		0.43	
contig13650:1:463:1  hypothetical protein BRAFLDRAFT_78483 [Branchiostoma floridae] (XP_002596622.1) Evalue: 2e-10	56	2	5.2	154		0.44	
contig10276:267:1127:3  secreted mucin MUC17, putative [Ixodes scapularis] (XP_002405770.1) Evalue: 7e-07	66	2	7	286		0.22	
contig07537:1:2003:1  hypothetical protein AaeL_AAEL002671 [Aedes aegypti] (XP_001662061.1) Evalue: 3e-47	26	1	1	667		0.08	
contig01805:541:1347:1  PREDICTED: similar to CG9896-PA [Apis mellifera] (XP_001121132.1) Evalue: 1e-12	36	1	2.2	268			0.3
contig07982:2215:3957:1  hypothetical protein AN3547 (XP_661151.1) Evalue: 6e-06	69	2	5.2	580			0.1
contig09488:1311:1508:1  endochitinase [Encephalitozoon cuniculi GB-M1] (XP_955684.1) Evalue: 4e-06	71	3 (2)	36.9	65			1.23
contig08882:53:733:2  putative B3/4 domain protein [Anaerobaculum hydrogeniformans ATCC BAA-1850] (ZP_06439514.1) Evalue: 4e-32	74	2	9.3	227			0.27
contig08804:1:508:2  PREDICTED: similar to ENSANGP00000008370 [Strongylocentrotus purpuratus] (XP_782704.1) Evalue: 3e-26	122	2	22.6	168			0.76
contig10251:1:692:3  oligopeptidase, putative [Ixodes scapularis] (XP_002405238.1) Evalue: 1e-15	41	1	5.2	229			0.26
contig06704:1:1179:1  Hypothetical protein CBG21994 [Caenorhabditis briggsae] (XP_002649060.1) Evalue: 3e-31	108	2	7.7	392			0.23
contig25674:77:1645:2  hypothetical protein SrosN1_01872 [Streptomyces roseosporus NRRL 11379] (ZP_04706698.1) Evalue: e-111	79	2	5	522			0.11
contig25155:1:869:3  AGAP002291-PA [Anopheles gambiae str (XP_307887.4) Evalue: 1e-08	166	3	13.2	288			0.34
contig20875:1:776:3 similar to FK506-binding protein 7 (Q5RET2) Evalue: 1e-39	386	10 (7)	40.9	257	2.69	1.14	1.66
contig21019:1:327:2 similar to FK506-binding protein 59 (Q9VL78) Evalue: 3e-16	67	2	19.4	108	4.35	0.61	
contig05000:114:947:3 similar to FK506-binding protein 4 (Q02790) Evalue: 3e-70	129	3	17.6	278	0.67	0.36	0.36
contig24369:128:1879:2 similar to FK506-binding protein 8 (Q14318) Evalue: 1e-30	152	4	6.5	583	0.2	0.16	0.32
contig24145:1:325:2 similar to 12 kDa FK506-binding protein (P48375) Evalue: 5e-42	315	9 (6)	64.5	107	5.05		
contig27480:1271:1948:2 similar to U2 small nuclear ribonucleoprotein B-- (Q9CQ17) Evalue: 1e-61	242	5	26.7	225	0.98		0.45
contig25934:101:1017:2 similar to Putative U5 small nuclear ribonucleoprotein 200 kDa helicase (Q9VUV9) Evalue: 2e-79	69	2	7.5	305	0.2		
contig23716:138:554:3 similar to Small nuclear ribonucleoprotein E (P62303) Evalue: 5e-31	101	3 (2)	15.2	138	0.98		
contig23467:1:232:2 similar to 33 kDa ribonucleoprotein, chloroplastic (P19684) Evalue: 9e-06	107	2	48.7	76			1.02
contig20197:1:310:2 similar to Small nuclear ribonucleoprotein F (Q24297) Evalue: 2e-30	195	3	20.6	102	2.38		
contig17765:1:364:3 similar to U2 small nuclear ribonucleoprotein A- (Q4R8Y8) Evalue: 2e-33	116	3	30.8	120	0.96		
contig13628:1:425:3 similar to Small nuclear ribonucleoprotein-associated protein B- (Q9N1Q0) Evalue: 6e-24	176	3 (2)	20.7	140	0.86	0.74	0.74
contig11272:1:2004:1 similar to Putative U5 small nuclear ribonucleoprotein 200 kDa helicase (Q9VUV9) Evalue: 0.0	175	4	7.3	667	0.22		
contig10302:2792:3055:2 similar to Probable small nuclear ribonucleoprotein G (Q9VXE0) Evalue: 4e-23	250	4 (3)	49.4	87	1.85		
contig09800:194:868:2 similar to U1 small nuclear ribonucleoprotein A (P09012) Evalue: 2e-51	86	2	14.3	224	0.28	0.28	
contig08017:96:325:3 similar to U2 small nuclear ribonucleoprotein A- (Q4R8Y8) Evalue: 3e-25	71	2	35.5	76	0.96		
contig07030:52:219:1 similar to H/ACA ribonucleoprotein complex subunit 3 (Q6DRH5) Evalue: 3e-19	113	3 (2)	45.5	55	4.3		
contig04387:1:491:3 similar to Probable small nuclear ribonucleoprotein Sm D2 (Q9VI10) Evalue: 2e-35	148	4	25.9	162	3.4	0.92	0.92
contig05743:1:1474:2 similar to H/ACA ribonucleoprotein complex subunit 4 (Q5ZJH9) Evalue: 9e-168	216	5	16.5	490		0.57	0.63
contig00939:1356:2102:2 similar to Peptide methionine sulfoxide reductase (P08761) Evalue: 3e-35	91	2	10.1	248	0.39	0.25	0.56

## Supplementary

contig00980:1:671:3 similar to Endoplasmic reticulum resident protein ERp44 (Q9BS26) Evalue: 4e-55	371	9 (8)	50	222	1.68	1.68	3.4
contig02178:397:983:1 similar to Endoplasmic reticulum resident protein ERp44 (Q3T0L2) Evalue: 5e-41	275	7 (6)	34.9	195	0.32	1.33	1.02
contig01054:1:615:1 similar to UPF0327 protein C1orf151 (Q5TGZ0) Evalue: 2e-19	197	5 (4)	21.1	204	0.84	2.3	4.73
contig27439:52:1863:1 similar to Signal recognition particle 68 kDa protein (Q9UHB9) Evalue: 3e-98	480	8	16.6	603		0.45	0.51
contig27375:1:181:3 similar to Signal recognition particle 54 kDa protein (P14576) Evalue: 6e-22	89	2	39	59		1.33	1.33
contig11237:1:893:3 similar to Signal recognition particle 72 kDa protein (P33731) Evalue: 4e-20	273	6	23.2	297	0.45	0.94	0.59
contig01242:93:365:3 similar to Signal recognition particle 14 kDa protein (P16254) Evalue: 5e-10	55	2	20	90	1.12		
contig01561:112:777:1 similar to Protein spaetzle (P48607) Evalue: 2e-05	106	2	12.7	221		0.28	0.65
contig26398:1:666:1 similar to Guanine nucleotide-binding protein subunit beta-2-like 1 (P68040) Evalue: 1e-69	447	17 (6)	34.8	221	3.89	2.96	4.07
contig24078:1:648:1 similar to Guanine nucleotide-binding protein subunit beta-2-like 1 (Q93134) Evalue: 1e-68	364	15 (7)	39.4	216	1.6	2.67	3.15
contig19762:7:1149:1 similar to Guanine nucleotide-binding protein G(s) subunit alpha (Q7PD79) Evalue: 6e-166	153	3	9.2	380		0.24	0.53
contig20740:157:963:1 similar to Guanine nucleotide-binding protein G(i) subunit alpha (P30676) Evalue: 2e-119	138	3	16	269	0.35		
contig25108:137:1204:2 similar to Guanine nucleotide-binding protein G(o) subunit alpha (P51877) Evalue: 0.0	333	9 (7)	23.1	355	0.36	1.03	0.47
contig19248:627:1691:3 similar to Guanine nucleotide-binding protein G(q) subunit alpha (Q28294) Evalue: 0.0	258	5	20.3	354	0.7	0.46	0.84
contig03131:1:907:2 similar to Guanine nucleotide-binding protein G(q) subunit alpha (P21279) Evalue: 7e-137	162	3	12.6	301	0.56	0.3	0.7
contig26477:1:1199:3 similar to Guanine nucleotide-binding protein G(l)/G(S)/G(T) subunit beta-1 (O45040) Evalue: 0.0	211	6 (5)	17.1	398	0.79	0.81	0.73
contig26338:1:1000:2 similar to Alpha-parvin (Q9NVD7) Evalue: 5e-102	402	9 (8)	27.4	332		1.28	1.48
contig26327:118:1743:1 similar to Golgi reassembly-stacking protein 2 (Q99JX3) Evalue: 1e-80	89	2	5	541	0.11	0.17	0.18
contig26271:55:1587:1 similar to ATP-binding cassette sub-family E member 1 (P61222) Evalue: 0.0	184	3	7.2	511	0.47	0.36	0.53
contig24341:1:224:1 similar to ATP-binding cassette sub-family E member 1 (P61222) Evalue: 1e-30	64	2	27	74	0.99		
contig23683:161:1192:2 similar to Protein NDRG3 (Q5RA95) Evalue: 2e-56	296	7 (5)	23.3	343	0.62	0.57	0.27
contig23669:1:371:1 similar to Tetratricopeptide repeat protein 19 (Q8CC21) Evalue: 1e-08	171	3	30.9	123	0.91	1.82	1.08
contig23648:380:1816:2 similar to Coronin-6 (Q920M5) Evalue: 4e-136	302	7	19.2	478	0.27	0.43	0.35
contig23622:1:2311:2 similar to Glycogen phosphorylase (Q9PKE6) Evalue: 0.0	1342	45 (28)	51.2	769	0.34	3.35	1.97
contig11894:71:359:2 similar to Glycogen phosphorylase, liver form (Q0VCM4) Evalue: 1e-37	186	3	35.4	96		1.19	0.99
contig26373:1:1159:2 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 9e-134	988	30 (21)	54.9	386	7.33	8.07	8.19
contig23734:1:153:2 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 6e-16	246	9 (4)	78	50	66.59	29.43	57.59
contig20201:1:273:1 similar to Transketolase-like protein 2 (Q9D4D4) Evalue: 5e-24	246	8 (3)	54.4	90	11.27	5.89	9.49
contig05600:1:307:2 similar to Transketolase (Q5R1W6) Evalue: 2e-12	45	1	10.8	102	0.6		1.22
contig28037:1:429:1 similar to Trifunctional enzyme subunit beta, mitochondrial (O46629) Evalue: 5e-60	259	8 (4)	38.5	143	3.91	4.4	2.52
contig27912:108:869:3 similar to Trifunctional enzyme subunit beta, mitochondrial (P55084) Evalue: 3e-81	381	10 (7)	29.5	254	1.87	3.25	1.58
contig27496:1:190:1 similar to Trifunctional enzyme subunit alpha, mitochondrial (P40939) Evalue: 6e-13	76	3 (1)	22.2	63	2.56	2.3	3.71
contig05463:1:187:1 similar to Trifunctional enzyme subunit beta, mitochondrial (Q60587) Evalue: 2e-12	125	3 (2)	40.3	62	2.77	1.98	4.86
contig01168:22:1775:1 similar to Trifunctional enzyme subunit alpha, mitochondrial (Q8BMS1) Evalue: 0.0	1107	29 (20)	51.9	584	1.81	3.95	3.08
contig01051:8:172:2 similar to Trifunctional enzyme subunit alpha, mitochondrial (Q64428) Evalue: 1e-12	123	4 (3)	42.6	54	2.59	2.85	2.85
contig19993:1:1042:2 similar to Trifunctional purine biosynthetic protein adenosine-3 (P21872) Evalue: 4e-65	291	6 (5)	21.3	347	1.33		
contig04427:1:1537:2 similar to Uridine 5~-monophosphate synthase (P31754) Evalue: 9e-100	199	4	11.5	511	0.86	0.25	0.32
contig02289:68:802:2 similar to Uridine 5~-monophosphate synthase (Q42942) Evalue: 9e-39	130	2	12.7	244		0.27	
contig05308:104:1705:2 similar to Bifunctional coenzyme A synthase (Q8MIR4) Evalue: 3e-77	89	2	4.1	533		0.11	
contig25413:56:228:2 similar to Rho GDP-dissociation inhibitor 1 (Q5XI73) Evalue: 7e-05	35	1	15.8	57	1.16		1.49
contig01847:1:417:1 similar to Rho GDP-dissociation inhibitor 2 (Q61599) Evalue: 4e-37	110	3	23.9	138	2.3	0.98	0.77

## Supplementary

contig21183:1:1353:1 similar to Rab GDP dissociation inhibitor beta (P50395) Evalue: 4e-165	1465	44 (25)	71.1	450	6.46	6.64	4.95
contig23612:1:901:2 similar to Apoptosis inhibitor 5 (Q5ZMW3) Evalue: 7e-39	132	3	12.7	300	0.46	0.32	0.32
contig23374:1:640:2 similar to Apoptosis inhibitor 5 homolog (Q9V431) Evalue: 1e-29	142	3	13.6	213	0.92	0.48	0.69
contig17836:1:706:1 similar to Probable Bax inhibitor 1 (Q9IA79) Evalue: 2e-47	116	3	13.6	235	0.43		
contig06974:219:1922:3 seems to belong to G-protein coupled receptor 2 family Best-hit: Gastric inhibitory polypeptide receptor (P48546) Evalue: 3e-05	522	15 (10)	26.5	567		0.92	0.66
contig24748:1:444:3 similar to Signal peptidase complex subunit 1 (Q5RF96) Evalue: 3e-06	120	4 (2)	16.3	147	0.63		
contig23795:1:487:2 similar to Probable signal peptidase complex subunit 2 (Q5BJI9) Evalue: 1e-27	149	4 (3)	30.4	161	0.66		
contig05652:1721:2191:1 similar to Probable signal peptidase complex subunit 2 (Q5M8Y1) Evalue: 3e-28	97	3 (2)	17.3	156		0.67	0.67
contig23304:1:554:3 similar to Signal peptidase complex subunit 3 (Q9VCA9) Evalue: 3e-33	185	6 (4)	23.5	183	1.62	0.98	0.98
contig27833:1:807:2 similar to Proteasome subunit beta type-4 (P99026) Evalue: 3e-61	211	5 (4)	26.1	268	0.94	0.77	0.7
contig26394:1:212:3 similar to 26S proteasome non-ATPase regulatory subunit 11 (Q2KI42) Evalue: 4e-17	279	5 (4)	76.8	69	11.32	6.12	6.12
contig25991:504:1997:3 similar to Probable 26S proteasome non-ATPase regulatory subunit 3 (O61470) Evalue: 9e-108	834	16 (14)	35.4	497	2.1	1.65	1.08
contig25696:1:661:3 similar to Proteasome subunit beta type-5 (P28075) Evalue: 1e-41	220	5 (3)	19.2	219	0.95	0.75	0.96
contig25245:117:283:3 similar to Proteasome subunit alpha type-7 (Q4R7D9) Evalue: 1e-20	84	2 (1)	40	55	2.16	3.12	2.16
contig24724:1:132:1 similar to Probable proteasome subunit alpha type-5 (Q9UT97) Evalue: 9e-12	128	2	50	44	2.51	2.51	3.19
contig24492:114:300:3 similar to Proteasome subunit beta type-3 (Q9XYN7) Evalue: 6e-14	52	2	29	62	1.36	1.36	
contig24290:1:322:1 similar to 26S proteasome non-ATPase regulatory subunit 14 (O76577) Evalue: 2e-58	55	1	24.3	107	0.58		0.66
contig23772:17:1069:2 similar to 26S proteasome non-ATPase regulatory subunit 13 (P84169) Evalue: 8e-65	398	10 (8)	31.1	351	1.58	1.12	1.19
contig23593:1:2727:1 similar to 26S proteasome regulatory subunit rpn1 (P87048) Evalue: 0.0	1392	28 (25)	38.9	908	1.71	1.61	1.38
contig23577:1:1110:2 similar to 26S proteasome non-ATPase regulatory subunit 11 (Q2KI42) Evalue: 1e-125	344	9 (7)	20.6	369	1.74	1.05	0.7
contig23554:1:932:3 similar to Proteasome subunit beta type-7 (Q9JHW0) Evalue: 4e-71	125	3	11	309	1.06	0.42	0.96
contig23497:1:1057:2 similar to 26S proteasome non-ATPase regulatory subunit 12 (O00232) Evalue: 4e-95	140	3	9.4	351	1.53	0.94	0.81
contig23476:1:1200:1 similar to 26S proteasome non-ATPase regulatory subunit 6 (Q15008) Evalue: 7e-121	289	6 (5)	14.8	399		0.64	
contig23476:1:1200:1 similar to 26S proteasome non-ATPase regulatory subunit 6 (Q15008) Evalue: 7e-121	378	10 (8)	23.6	399	1.26		0.67
contig23427:1:813:1 similar to Proteasome subunit beta type-6 (Q3MHN0) Evalue: 1e-64	406	9 (8)	39.6	270	2.22	1.39	2.14
contig23393:1:2252:3 similar to 26S proteasome non-ATPase regulatory subunit 1 (Q5R5S4) Evalue: 0.0	435	12 (8)	16.8	749	0.37	0.67	0.44
contig23084:1696:2436:2 similar to Proteasome subunit alpha type-6 (Q9QUM9) Evalue: 1e-98	221	6	32.1	246	1.51	1.59	0.52
contig22991:1:525:2 similar to Proteasome subunit alpha type-1 (Q9R1P4) Evalue: 7e-53	497	16 (10)	75.3	174	5.8	6.88	6.02
contig22869:1:714:1 similar to Proteasome subunit beta type-2 (Q5E9K0) Evalue: 2e-53	386	10 (6)	40.9	237	1.53	2.16	1.89
contig22783:1:612:1 similar to Proteasome subunit alpha type-5 (Q5E987) Evalue: 2e-82	541	13 (10)	81.8	203	3.15	4.8	2.55
contig22702:1:727:3 similar to 26S proteasome non-ATPase regulatory subunit 8 (Q5RE15) Evalue: 2e-48	415	13 (8)	37.8	241	2.19	1.9	1.9
contig20565:1:515:3 similar to 26S proteasome non-ATPase regulatory subunit 14 (Q9V3H2) Evalue: 2e-77	67	3 (2)	11.8	170	1.52	0.38	0.62
contig20397:1:507:2 similar to Proteasome activator complex subunit 4 (Q6NRP2) Evalue: 2e-18	82	3 (2)	11.9	168		0.57	0.39
contig20097:1:284:2 similar to 26S proteasome non-ATPase regulatory subunit 12 (Q2KJ25) Evalue: 2e-15	59	1	12.8	94	0.99	0.66	0.66
contig19953:1:431:3 similar to Proteasome subunit beta type-3 (O73817) Evalue: 4e-51	96	5 (2)	12	142	0.94	0.68	0.94
contig19226:156:932:3 similar to 26S proteasome non-ATPase regulatory subunit 10 (Q9Z2X2) Evalue: 8e-50	157	5 (4)	18.6	258	0.5	0.56	0.39
contig18594:48:710:3 similar to Proteasome subunit beta type-1-B (Q9IB83) Evalue: 2e-51	444	12 (8)	50.9	220	2.07	2.56	2.37
contig18219:1:768:1 similar to Proteasome subunit alpha type-3 (P25788) Evalue: 1e-89	627	15 (11)	43.5	255	2.74	2.77	3.37
contig17993:1:578:3 similar to Proteasome subunit alpha type-7-like (Q9CWH6) Evalue: 3e-65	292	8 (6)	46.1	191	1.22	1.22	1.9
contig08368:229:1212:1 similar to 26S proteasome non-ATPase regulatory subunit 7 (P26270) Evalue: 9e-99	447	11 (9)	42.2	327	2.49	1.45	1.81
contig06946:143:937:2 similar to Proteasome subunit alpha type-4 (P25789) Evalue: 1e-97	323	9 (7)	37.1	264	0.81	1.75	2.21

## Supplementary

contig06282:1:1063:2 similar to 26S proteasome non-ATPase regulatory subunit 4 (P55036) Evalue: 4e-97	238	3	14.4	353	0.67	0.46	0.27
contig03291:1:301:2 similar to Proteasome subunit beta type-8 (P28063) Evalue: 2e-23	266	10 (4)	52.5	99	4.22	3.92	7.68
contig02388:1:106:2 similar to Proteasome subunit alpha type-2 (Q3T0Y5) Evalue: 4e-07	58	2 (1)	31.4	35		2.14	2.14
contig00616:1:522:1 similar to Proteasome subunit alpha type-2 (O73672) Evalue: 1e-65	354	10 (5)	46.2	173	2.65	3.72	1.77
contig22885:265:2031:1 similar to Syntaxin-binding protein 1 (P61765) Evalue: 0.0	367	8	17.3	588	0.1	0.61	0.6
contig18115:1:705:1 similar to Syntaxin-12 (Q9ER00) Evalue: 8e-12	247	7 (5)	25.1	235	0.9	0.9	1
contig02803:1:176:3 similar to Syntaxin-18 (Q8VDS8) Evalue: 1e-11	26	1	21.1	57		1.08	1.08
contig24979:203:1807:2 similar to Lamin Dm0 (P08928) Evalue: 5e-28	307	5	11.6	534	3.63	0.34	0.76
contig20808:1:1714:2 similar to Laminin subunit beta-1 (P02469) Evalue: 2e-07	752	14 (13)	33	570	0.15	1.12	0.61
contig18777:1:1452:1 similar to Laminin subunit beta-1 (P11046) Evalue: 4e-40	323	6	13.5	483		0.55	0.61
contig17793:1:1217:3 similar to Laminin subunit alpha-4 (Q16363) Evalue: 2e-58	590	11 (9)	31.7	404	0.22	1.26	0.92
contig16187:1:682:2 similar to Laminin subunit gamma-3 (Q9R0B6) Evalue: 7e-69	99	2	8.4	227		0.27	
contig16148:1:480:1 similar to Laminin subunit gamma-1 (A0JP86) Evalue: 1e-15	63	2	11.2	160	0.41		0.38
contig14260:156:1061:3 similar to Laminin subunit beta-2 (Q61292) Evalue: 3e-93	204	6 (4)	18.3	301	0.31	1.17	0.57
contig07950:1:943:1 similar to Laminin subunit gamma-1 (P15215) Evalue: 6e-22	472	7 (6)	29.6	314	0.59	0.94	0.74
contig03109:97:1837:1 similar to Lamin Dm0 (P08928) Evalue: 4e-60	1631	50 (31)	56.6	580	2.59	5.88	3.37
contig12174:1:369:1 similar to Filamin-B (Q80X90) Evalue: 4e-32	189	3	35.2	122		3.03	2.15
contig10543:1:889:2 similar to Filamin-A (Q8BTM8) Evalue: 1e-72	1124	48 (18)	73.2	295	1.35	36.73	32.72
contig10542:1:699:1 similar to Filamin-C (Q8VHX6) Evalue: 2e-49	348	11 (5)	33.5	233		4.4	4.67
contig25243:1:1182:1 similar to Ubiquitin-like modifier-activating enzyme 1 (Q02053) Evalue: 4e-137	356	9 (7)	24.2	393	0.4	0.83	0.39
contig24694:1426:2364:1 similar to Ubiquitin fusion degradation protein 1 homolog (Q9ES53) Evalue: 1e-88	138	3	11.9	312	0.31		0.2
contig22931:236:1744:2 similar to Ubiquitin carboxyl-terminal hydrolase 10 (Q14694) Evalue: 3e-64	67	2	4.6	502		0.12	
contig20864:244:620:1 similar to Probable ubiquitin-conjugating enzyme E2 W (Q96B02) Evalue: 1e-33	73	2	20	125	0.55		
contig20519:78:413:3 similar to Probable ubiquitin-fold modifier 1 (P34661) Evalue: 7e-38	133	3 (2)	42.3	111	0.96		
contig19867:750:1022:3 similar to Ubiquitin domain-containing protein UBFD1 (O14562) Evalue: 2e-33	88	2	42.2	90	0.76		
contig19867:214:744:1 similar to Ubiquitin domain-containing protein UBFD1 (O14562) Evalue: 3e-20	176	4 (3)	26.1	176	0.63	0.92	0.39
contig19281:1:641:3 similar to Ubiquitin-conjugating enzyme E2 L3 (Q5R5I4) Evalue: 3e-67	153	5 (3)	22.6	212	0.46		
contig10373:377:943:2 similar to Ubiquitin-conjugating enzyme E2 H (P62257) Evalue: 2e-73	79	2	9.6	188	0.33		
contig10326:1:1355:3 similar to Ubiquitin carboxyl-terminal hydrolase 7 (Q4VSI4) Evalue: 8e-80	154	4	9.6	450	0.34		
contig10252:1:1867:1 similar to Ubiquitin carboxyl-terminal hydrolase 15 (Q9Y4E8) Evalue: 3e-74	66	2	3.9	622		0.1	
contig10062:1:1118:1 similar to Ubiquitin-like modifier-activating enzyme 1 (Q02053) Evalue: 7e-132	358	6	25	372	0.16	1.22	0.67
contig08583:572:2251:2 similar to Ubiquitin carboxyl-terminal hydrolase 5 (P45974) Evalue: 2e-123	105	3	5	559	0.11	0.16	
contig08583:1:568:2 similar to Ubiquitin carboxyl-terminal hydrolase 5 (P56399) Evalue: 1e-53	85	2	15.4	188	0.35	0.51	
contig08449:115:1263:1 similar to Proteasomal ubiquitin receptor ADRM1 (Q98SH3) Evalue: 4e-39	417	9	35.3	382	0.72	1.01	0.59
contig06315:174:1087:3 similar to Ubiquitin carboxyl-terminal hydrolase isozyme L5 (Q9XSJ0) Evalue: 5e-94	153	4	12.8	304	0.53	0.53	0.81
contig06136:1:520:2 similar to Ubiquitin carboxyl-terminal hydrolase 14 (Q0IIF7) Evalue: 2e-21	100	3	26.2	172	0.37	0.78	0.34
contig05452:116:718:2 similar to Ubiquitin-conjugating enzyme E2 K (P61085) Evalue: 2e-55	121	2	16	200	0.32	0.32	0.32
contig03967:1:1305:1 similar to Ubiquitin-like modifier-activating enzyme 5 (Q5R8X4) Evalue: 7e-120	31	1	2.1	434	0.29	0.14	0.29
contig03560:140:1163:2 similar to Ubiquitin-associated protein 2-like (Q80X50) Evalue: 2e-12	266	4	20.8	341	0.5		
contig02744:216:1373:3 similar to Ubiquitin-conjugating enzyme E2 Q2 (Q8WVN8) Evalue: 5e-63	89	2	7.3	385			0.16
contig01819:114:1244:3 similar to Ubiquitin thioesterase otubain-like (Q9VL00) Evalue: 2e-61	115	3	8.5	376	0.34	0.25	0.25

## Supplementary

contig01677:1:482:1 similar to Ubiquitin-conjugating enzyme E2-17 kDa (P25867) Evalue: 2e-52	67	2	11.2	160	0.6			
contig01629:164:487:2 similar to Ubiquitin-conjugating enzyme E2-24 kDa (P52485) Evalue: 4e-20	27	1	6.5	108				1.38
contig00934:1:168:1 similar to Ubiquitin-conjugating enzyme E2 N (Q0P5K3) Evalue: 2e-17	77	2 (1)	20	55	1.18			
contig00732:552:995:3 similar to Ubiquitin-conjugating enzyme E2 variant 2 (Q7M767) Evalue: 3e-58	196	7 (5)	34	147	2.24			
contig00633:1:356:1 similar to Ubiquitin (P68195) Evalue: 3e-33	115	3 (2)	21.2	118	15.03	6.72		10.18
contig00319:200:508:2 similar to Ubiquitin-conjugating enzyme E2 N (P35128) Evalue: 4e-45	137	4 (3)	52.4	103	2.38			
contig02068:1:810:3 similar to Importin-5 (O00410) Evalue: 2e-47	306	5	29.4	269	0.51	1.72		1.19
contig26217:1:626:3 similar to Importin-5 (O00410) Evalue: 8e-37	319	7 (6)	35.7	207	0.77	2.02		0.6
contig19296:1:573:1 similar to Importin subunit beta-1 (Q14974) Evalue: 1e-22	103	2	13.7	190	0.35			
contig10183:626:2053:2 similar to Importin subunit beta (O18388) Evalue: 5e-92	450	9 (8)	24.6	476	0.6			0.51
contig09965:1:1632:1 similar to Importin subunit alpha-7 (O35345) Evalue: 2e-165	495	9 (8)	27.3	543	1.54	1.06		1.77
contig07267:1:3001:2 similar to Importin-8 (O15397) Evalue: 0.0	305	6	9.9	999	0.25	0.18		0.09
contig26298:1:343:3 similar to Ran-binding protein 17 (Q9H2T7) Evalue: 2e-14	44	1	8	113	0.84	0.52		
contig28085:152:553:2 similar to B-cell receptor-associated protein 31 (P51572) Evalue: 1e-17	127	7 (2)	20.1	134	1.72	2.33		2.15
contig26550:1:288:1 similar to B-cell receptor-associated protein 31 (Q5R8H3) Evalue: 4e-06	226	9 (4)	40	95	6.42	6.18		5.61
contig18325:113:1433:2 similar to Programmed cell death protein 4 (Q98TX3) Evalue: 1e-56	559	14 (10)	31.1	440	2.22	1.32		1.55
contig12755:145:2718:1 similar to Programmed cell death 6-interacting protein (Q8WUM4) Evalue: 2e-179	924	18	25.3	857	1.26	1		0.74
contig01090:361:506:3 similar to Programmed cell death protein 4 (Q53EL6) Evalue: 6e-06	132	3	63.8	47	1.94			
contig01001:118:678:1 similar to Programmed cell death protein 5 (P56812) Evalue: 7e-21	33	1	5.9	186	0.32			
contig27971:1:261:1 similar to Malignant T cell amplified sequence 1 (Q6DER1) Evalue: 9e-30	92	1	22.1	86				
contig27966:1:312:1 similar to Receptor expression-enhancing protein 5 (Q60870) Evalue: 1e-14	90	5 (2)	17.5	103	5.31	3.58		2.59
contig00276:121:418:1 similar to Receptor expression-enhancing protein 5 (B2RZ37) Evalue: 4e-10	193	6 (5)	39.4	99	10.59	3.6		5.72
contig23065:1:691:1 similar to Acidic leucine-rich nuclear phosphoprotein 32 family member A (P51122) Evalue: 3e-32	122	3	26.1	230	0.81			0.26
contig16533:1:782:3 similar to Stress-induced-phosphoprotein 1 (Q4R8N7) Evalue: 7e-84	391	9 (8)	28.2	259	0.89	1.1		1.34
contig00707:1:1070:1 similar to Stress-induced-phosphoprotein 1 (Q4R8N7) Evalue: 6e-43	385	6	21.9	356	0.64	0.64		0.78
contig05513:566:1825:2 similar to Golgi phosphoprotein 3 (Q9H4A6) Evalue: 8e-76	292	4	13.1	419	0.49	0.37		0.39
contig02325:22:654:1 similar to 28 kDa heat- and acid-stable phosphoprotein (Q13442) Evalue: 1e-04	131	3	14.8	210	1.94	0.49		0.71
contig00027:1:1861:2 similar to Caprin-2 (Q6IMN6) Evalue: 1e-12	107	2	5.6	620	0.26	0.15		0.26
contig27705:858:2429:3 similar to Nose resistant to fluoxetine protein 6 (Q09225) Evalue: 5e-41	332	6	12.4	523	0.65	1.47		0.97
contig27705:424:804:1 similar to Nose resistant to fluoxetine protein 6 (Q09225) Evalue: 1e-07	102	2	16.7	126	0.46	0.75		1.27
contig27458:1:2039:3 similar to Dynamin-1-like protein (Q7SXN5) Evalue: 3e-76	1045	26 (16)	33.8	678		3.09		2.13
contig09722:1:1670:3 similar to Dynamin-1-like protein (Q7SXN5) Evalue: 0.0	381	6	14.4	555	0.51	0.36		0.17
contig09682:1:1043:3 similar to Talin-1 (Q9Y490) Evalue: 2e-55	166	3	12.1	346		1.46		0.91
contig03404:110:835:2 similar to Translin (P79769) Evalue: 3e-48	144	3	16.2	241	0.41	0.41		0.41
contig18560:105:292:3 similar to GTP-binding nuclear protein Ran (Q9VZ23) Evalue: 2e-18	71	2	30.6	62	2.49	1.3		1.04
contig02640:1:442:2 similar to GTP-binding nuclear protein Ran (P38542) Evalue: 9e-69	225	7 (5)	41.1	146	2.39	1.93		2.37
contig21564:1:607:2 similar to Microtubule-associated proteins 1A/1B light chain 3C (Q9BXW4) Evalue: 3e-34	64	2	11.9	201	0.58			
contig01656:139:1230:1 similar to Microtubule-associated protein RP/EB family member 1 (Q61166) Evalue: 3e-47	194	3	15.4	363	0.63	0.28		0.5
contig26776:1:590:3 similar to Translocon-associated protein subunit gamma (Q08013) Evalue: 2e-50	134	2	7.2	195	1.09	0.62		0.77
contig24346:41:685:2 similar to Translocon-associated protein subunit alpha (P43307) Evalue: 5e-30	221	8 (3)	20	215	1.12	1.9		1.3
contig24227:1:649:2 similar to Translocon-associated protein subunit delta (Q62186) Evalue: 6e-14	50	1	6	215	3.86	0.28		0.3

contig00145:104:676:2 similar to Translocon-associated protein subunit beta (Q5E9E4) Evalue: 1e-41	116	4 (2)	21.1	190	0.5	0.71	0.68
contig00237:1:1696:2 similar to Fragile X mental retardation protein 1 homolog B (Q2KHP9) Evalue: 2e-25	124	2	6	564	0.35	0.22	0.22
contig23845:1:545:3 similar to Coiled-coil domain-containing protein 25 (Q78PG9) Evalue: 5e-37	102	2	12.2	180	0.68	0.34	0.34
contig00396:125:1666:2 similar to Coiled-coil domain-containing protein 47 (Q3ZC50) Evalue: 5e-80	64	2	4.3	513	0.18	0.11	
contig18998:1:538:2 similar to Signal recognition particle receptor subunit beta (P47758) Evalue: 5e-19	194	4	28.7	178	0.35	1.66	0.93
contig00643:105:1985:3 similar to Signal recognition particle receptor subunit alpha homolog (Q9U5L1) Evalue: 1e-162	571	11	25.9	626	0.1	0.7	0.57
contig26949:108:641:3 similar to Bifunctional protein glmU (Q2JVA4) Evalue: 4e-20	304	4 (3)	30.5	177	0.38	0.9	0.9
contig00472:1:1212:1 similar to Bifunctional protein NCOAT (Q9EQQ9) Evalue: 8e-57	27	1	1.7	403			0
contig01241:46:975:1 similar to Protein henna (P17276) Evalue: 6e-83	434	9 (8)	37.4	310	0.57	1.05	1.24
contig11949:210:608:3 similar to Lambda-crystallin homolog (Q5RDZ2) Evalue: 1e-24	95	2	15.8	133		0.75	0.52
contig01555:212:841:2 similar to S-crystallin 4 (P27012) Evalue: 3e-25	564	20 (11)	48.8	209	1.17	3.59	3.26
contig01027:1:528:1 similar to Lambda-crystallin homolog (Q8SPX7) Evalue: 2e-44	238	4 (3)	26.3	175		0.6	0.88
contig00465:111:1184:3 similar to Serum paraoxonase/arylesterase 1 (P27169) Evalue: 2e-48	763	31 (13)	44.8	357	5.04	6.75	6.4
contig00426:1:447:1 similar to 6-phosphogluconolactonase (Q2TBQ8) Evalue: 8e-39	135	3	47.3	148	0.42	0.75	1.12
contig17900:249:560:3 similar to 6-phosphogluconolactonase (O95336) Evalue: 1e-13	225	5 (4)	56.7	104		2.81	0.95
contig00328:121:1038:1 similar to Mitochondrial import receptor subunit TOM40 homolog 1 (Q9U4L6) Evalue: 2e-90	573	10 (9)	36.4	305	1.15	1.97	1.11
contig11595:1:1582:2 similar to Mitochondrial import receptor subunit TOM70 (Q75Q39) Evalue: 6e-57	351	8 (7)	16.3	526	0.39	0.6	0.39
contig01412:1:447:1 similar to Mitochondrial import receptor subunit TOM20 homolog (A6H7B1) Evalue: 3e-16	303	9 (5)	44.6	148	2.16		
contig20884:1727:2107:1 similar to Mitochondrial import receptor subunit TOM22 homolog (Q9CPQ3) Evalue: 2e-09	206	3	46.8	126	0.96		
contig00317:1:1870:2 No distinct annotation Best-hit: Chromo domain-containing protein 1 (Q10103) Evalue: 1e-07	170	4	7.9	622	0.31		
contig07180:264:860:3 similar to COP9 signalosome complex subunit 4 (Q68FS2) Evalue: 4e-31	109	2	12.6	199	0.3	0.32	0.32
contig03885:82:1348:1 similar to COP9 signalosome complex subunit 2 (Q6IR75) Evalue: 3e-154	110	3	8.8	422	0.14	0.22	0.22
contig00295:1:663:1 similar to COP9 signalosome complex subunit 4 (Q9V345) Evalue: 3e-52	196	5	30	220	0.77	0.86	0.45
contig22596:73:843:1 similar to COP9 signalosome complex subunit 7a (Q5R762) Evalue: 2e-22	152	4 (3)	14.5	256	1.08	0.38	0.38
contig18729:1:563:3 similar to COP9 signalosome complex subunit 6 (Q9VVCY3) Evalue: 4e-10	103	2	13.4	186	0.16	0.35	
contig05329:1:772:2 similar to COP9 signalosome complex subunit 5 (Q9XZ58) Evalue: 9e-86	157	3	15.2	257	0.38	0.38	
contig01155:1532:2998:2 similar to COP9 signalosome complex subunit 1 (Q6NRT5) Evalue: 1e-70	232	5	17	489	0.12	0.33	
contig22986:1:473:3 similar to COP9 signalosome complex subunit 5 (Q9XZ58) Evalue: 8e-11	192	3	27.6	156	0.68		
contig00201:1:1241:2 similar to 5-oxoprolinase (O14841) Evalue: 8e-141	84	2	6.1	413		0.15	0.24
contig00109:295:1377:1 similar to Angiotensin-like protein 1 (Q8IY63) Evalue: 4e-21	33	1	1.7	360		0.16	
contig00068:1:1403:3 similar to Propionyl-CoA carboxylase beta chain, mitochondrial (Q2TBR0) Evalue: 0.0	331	7 (6)	15.7	466		0.52	0.99
similar to Baculoviral IAP repeat-containing protein 7-A (Q8JHV9) Evalue: 8e-04	27	2 (1)	1.6	369	0.08		
contig19865:1:1110:3 similar to Baculoviral IAP repeat-containing protein 7-A (Q8JHV9) Evalue: 8e-04	27	2 (1)	1.6	369		0.16	
contig27315:1:1930:2 similar to Ran GTPase-activating protein (Q9VIW3) Evalue: 3e-42	307	6	11.5	642	0.71	0.3	0.36
contig26652:150:1358:3 similar to Meprin A subunit alpha (Q16819) Evalue: 5e-36	223	7 (4)	11.7	402	0.32	0.41	0.51
contig27718:205:957:1 similar to EF-hand domain-containing protein D2 (Q9D8Y0) Evalue: 1e-61	173	4	20	250	0.55		0.39
contig19939:1:306:1 similar to LETM1 and EF-hand domain-containing protein 1, mitochondrial (Q5ZK33) Evalue: 4e-12	237	5	58.4	101		3.38	1.38
contig09467:128:975:2 similar to LETM1 and EF-hand domain-containing protein 1, mitochondrial (Q0VA06) Evalue: 5e-47	164	2	11	282		0.31	0.68
contig09363:67:706:1 similar to Carbohydrate kinase domain-containing protein (Q9CZ42) Evalue: 2e-66	88	2	9.9	213	1.07	0.3	0.44
contig23362:342:2066:3 similar to FGGY carbohydrate kinase domain-containing protein (Q6DCD1) Evalue: 8e-117	315	5	12.5	574	0.1	0.27	0.33
contig23482:88:621:1 similar to Transcription factor BTF3 homolog (Q18885) Evalue: 6e-25	412	9 (8)	40.7	177	2.15	3.4	1.6



contig27971:1:261:1 similar to Malignant T cell amplified sequence 1 (Q6DER1) Evalue: 9e-30	92	1	22.1	86				
contig27966:1:312:1 similar to Receptor expression-enhancing protein 5 (Q60870) Evalue: 1e-14	90	5 (2)	17.5	103	5.31	3.58	2.59	
contig00276:121:418:1 similar to Receptor expression-enhancing protein 5 (B2RZ37) Evalue: 4e-10	193	6 (5)	39.4	99	10.59	3.6	5.72	
contig23065:1:691:1 similar to Acidic leucine-rich nuclear phosphoprotein 32 family member A (P51122) Evalue: 3e-32	122	3	26.1	230	0.81		0.26	
contig16533:1:782:3 similar to Stress-induced-phosphoprotein 1 (Q4R8N7) Evalue: 7e-84	391	9 (8)	28.2	259	0.89	1.1	1.34	
contig00707:1:1070:1 similar to Stress-induced-phosphoprotein 1 (Q4R8N7) Evalue: 6e-43	385	6	21.9	356	0.64	0.64	0.78	
contig05513:566:1825:2 similar to Golgi phosphoprotein 3 (Q9H4A6) Evalue: 8e-76	292	4	13.1	419	0.49	0.37	0.39	
contig02325:22:654:1 similar to 28 kDa heat- and acid-stable phosphoprotein (Q13442) Evalue: 1e-04	131	3	14.8	210	1.94	0.49	0.71	
contig00027:1:1861:2 similar to Caprin-2 (Q6IMN6) Evalue: 1e-12	107	2	5.6	620	0.26	0.15	0.26	
contig27705:858:2429:3 similar to Nose resistant to fluoxetine protein 6 (Q09225) Evalue: 5e-41	332	6	12.4	523	0.65	1.47	0.97	
contig27705:424:804:1 similar to Nose resistant to fluoxetine protein 6 (Q09225) Evalue: 1e-07	102	2	16.7	126	0.46	0.75	1.27	
contig27458:1:2039:3 similar to Dynamin-1-like protein (Q7SXX5) Evalue: 3e-76	1045	26 (16)	33.8	678		3.09	2.13	
contig09722:1:1670:3 similar to Dynamin-1-like protein (Q7SXX5) Evalue: 0.0	381	6	14.4	555	0.51	0.36	0.17	
contig09682:1:1043:3 similar to Talin-1 (Q9Y490) Evalue: 2e-55	166	3	12.1	346		1.46	0.91	
contig03404:110:835:2 similar to Translin (P79769) Evalue: 3e-48	144	3	16.2	241	0.41	0.41	0.41	
contig18560:105:292:3 similar to GTP-binding nuclear protein Ran (Q9VZ23) Evalue: 2e-18	71	2	30.6	62	2.49	1.3	1.04	
contig02640:1:442:2 similar to GTP-binding nuclear protein Ran (P38542) Evalue: 9e-69	225	7 (5)	41.1	146	2.39	1.93	2.37	
contig21564:1:607:2 similar to Microtubule-associated proteins 1A/1B light chain 3C (Q9BXW4) Evalue: 3e-34	64	2	11.9	201	0.58			
contig01656:139:1230:1 similar to Microtubule-associated protein RP/EB family member 1 (Q61166) Evalue: 3e-47	194	3	15.4	363	0.63	0.28	0.5	
contig26776:1:590:3 similar to Translocon-associated protein subunit gamma (Q08013) Evalue: 2e-50	134	2	7.2	195	1.09	0.62	0.77	
contig24346:41:685:2 similar to Translocon-associated protein subunit alpha (P43307) Evalue: 5e-30	221	8 (3)	20	215	1.12	1.9	1.3	
contig24227:1:649:2 similar to Translocon-associated protein subunit delta (Q62186) Evalue: 6e-14	50	1	6	215	3.86	0.28	0.3	
contig00145:104:676:2 similar to Translocon-associated protein subunit beta (Q5E9E4) Evalue: 1e-41	116	4 (2)	21.1	190	0.5	0.71	0.68	
contig00237:1:1696:2 similar to Fragile X mental retardation protein 1 homolog B (Q2KHP9) Evalue: 2e-25	124	2	6	564	0.35	0.22	0.22	
contig23845:1:545:3 similar to Coiled-coil domain-containing protein 25 (Q78PG9) Evalue: 5e-37	102	2	12.2	180	0.68	0.34	0.34	
contig00396:125:1666:2 similar to Coiled-coil domain-containing protein 47 (Q3ZC50) Evalue: 5e-80	64	2	4.3	513	0.18	0.11		
contig18998:1:538:2 similar to Signal recognition particle receptor subunit beta (P47758) Evalue: 5e-19	194	4	28.7	178	0.35	1.66	0.93	
contig00643:105:1985:3 similar to Signal recognition particle receptor subunit alpha homolog (Q9U5L1) Evalue: 1e-162	571	11	25.9	626	0.1	0.7	0.57	
contig26949:108:641:3 similar to Bifunctional protein glmU (Q2JVA4) Evalue: 4e-20	304	4 (3)	30.5	177	0.38	0.9	0.9	
contig00472:1:1212:1 similar to Bifunctional protein NCOAT (Q9EQQ9) Evalue: 8e-57	27	1	1.7	403			0	
contig01241:46:975:1 similar to Protein henna (P17276) Evalue: 6e-83	434	9 (8)	37.4	310	0.57	1.05	1.24	
contig11949:210:608:3 similar to Lambda-crystallin homolog (Q5RDZ2) Evalue: 1e-24	95	2	15.8	133		0.75	0.52	
contig01555:212:841:2 similar to S-crystallin 4 (P27012) Evalue: 3e-25	564	20 (11)	48.8	209	1.17	3.59	3.26	
contig01027:1:528:1 similar to Lambda-crystallin homolog (Q8SPX7) Evalue: 2e-44	238	4 (3)	26.3	175		0.6	0.88	
contig00465:111:1184:3 similar to Serum paraoxonase/arylesterase 1 (P27169) Evalue: 2e-48	763	31 (13)	44.8	357	5.04	6.75	6.4	
contig00426:1:447:1 similar to 6-phosphogluconolactonase (Q2TBQ8) Evalue: 8e-39	135	3	47.3	148	0.42	0.75	1.12	
contig17900:249:560:3 similar to 6-phosphogluconolactonase (O95336) Evalue: 1e-13	225	5 (4)	56.7	104		2.81	0.95	
contig00328:121:1038:1 similar to Mitochondrial import receptor subunit TOM40 homolog 1 (Q9U4L6) Evalue: 2e-90	573	10 (9)	36.4	305	1.15	1.97	1.11	
contig11595:1:1582:2 similar to Mitochondrial import receptor subunit TOM70 (Q75Q39) Evalue: 6e-57	351	8 (7)	16.3	526	0.39	0.6	0.39	
contig01412:1:447:1 similar to Mitochondrial import receptor subunit TOM20 homolog (A6H7B1) Evalue: 3e-16	303	9 (5)	44.6	148	2.16			
contig20884:1727:2107:1 similar to Mitochondrial import receptor subunit TOM22 homolog (Q9CPQ3) Evalue: 2e-09	206	3	46.8	126	0.96			

contig00317:1:1870:2 No distinct annotation Best-hit: Chromo domain-containing protein 1 (Q10103) Evalue: 1e-07	170	4	7.9	622	0.31			
contig07180:264:860:3 similar to COP9 signalosome complex subunit 4 (Q68FS2) Evalue: 4e-31	109	2	12.6	199	0.3	0.32	0.32	
contig03885:82:1348:1 similar to COP9 signalosome complex subunit 2 (Q6IR75) Evalue: 3e-154	110	3	8.8	422	0.14	0.22	0.22	
contig00295:1:663:1 similar to COP9 signalosome complex subunit 4 (Q9V345) Evalue: 3e-52	196	5	30	220	0.77	0.86	0.45	
contig22596:73:843:1 similar to COP9 signalosome complex subunit 7a (Q5R762) Evalue: 2e-22	152	4 (3)	14.5	256	1.08	0.38	0.38	
contig18729:1:563:3 similar to COP9 signalosome complex subunit 6 (Q9VVCY3) Evalue: 4e-10	103	2	13.4	186	0.16	0.35		
contig05329:1:772:2 similar to COP9 signalosome complex subunit 5 (Q9XZ58) Evalue: 9e-86	157	3	15.2	257	0.38	0.38		
contig01155:1532:2998:2 similar to COP9 signalosome complex subunit 1 (Q6NRT5) Evalue: 1e-70	232	5	17	489	0.12	0.33		
contig22986:1:473:3 similar to COP9 signalosome complex subunit 5 (Q9XZ58) Evalue: 8e-11	192	3	27.6	156	0.68			
contig00201:1:1241:2 similar to 5-oxoprolinase (O14841) Evalue: 8e-141	84	2	6.1	413		0.15	0.24	
contig00109:295:1377:1 similar to Angiotenin-like protein 1 (Q8IY63) Evalue: 4e-21	33	1	1.7	360		0.16		
contig00068:1:1403:3 similar to Propionyl-CoA carboxylase beta chain, mitochondrial (Q2TBR0) Evalue: 0.0	331	7 (6)	15.7	466		0.52	0.99	
similar to Baculoviral IAP repeat-containing protein 7-A (Q8JHV9) Evalue: 8e-04	27	2 (1)	1.6	369	0.08			
contig19865:1:1110:3 similar to Baculoviral IAP repeat-containing protein 7-A (Q8JHV9) Evalue: 8e-04	27	2 (1)	1.6	369		0.16		
contig27315:1:1930:2 similar to Ran GTPase-activating protein (Q9VIW3) Evalue: 3e-42	307	6	11.5	642	0.71	0.3	0.36	
contig26652:150:1358:3 similar to Meprin A subunit alpha (Q16819) Evalue: 5e-36	223	7 (4)	11.7	402	0.32	0.41	0.51	
contig27718:205:957:1 similar to EF-hand domain-containing protein D2 (Q9D8Y0) Evalue: 1e-61	173	4	20	250	0.55		0.39	
contig19939:1:306:1 similar to LETM1 and EF-hand domain-containing protein 1, mitochondrial (Q5ZK33) Evalue: 4e-12	237	5	58.4	101		3.38	1.38	
contig09467:128:975:2 similar to LETM1 and EF-hand domain-containing protein 1, mitochondrial (Q0VA06) Evalue: 5e-47	164	2	11	282		0.31	0.68	
contig09363:67:706:1 similar to Carbohydrate kinase domain-containing protein (Q9CZ42) Evalue: 2e-66	88	2	9.9	213	1.07	0.3	0.44	
contig23362:342:2066:3 similar to FGGY carbohydrate kinase domain-containing protein (Q6DCD1) Evalue: 8e-117	315	5	12.5	574	0.1	0.27	0.33	
contig23482:88:621:1 similar to Transcription factor BTF3 homolog (Q18885) Evalue: 6e-25	412	9 (8)	40.7	177	2.15	3.4	1.6	
contig23443:153:623:3 similar to V-type proton ATPase 16 kDa proteolipid subunit (P63081) Evalue: 2e-53	38	1	11.5	156	1.15			
contig24386:111:992:3 similar to V-type proton ATPase subunit d (Q25531) Evalue: 8e-127	254	6	20.4	294	0.76	0.76	0.86	
contig22339:27:1898:3 similar to V-type proton ATPase catalytic subunit A (Q90647) Evalue: 0.0	1543	40 (28)	55.5	623	1.96	4.05	3.15	
contig27950:148:1366:1 similar to Probable V-type proton ATPase subunit B (Q19626) Evalue: 0.0	897	23 (14)	55.2	406	1.93	3.51	3.6	
contig21495:234:1124:3 similar to V-type proton ATPase subunit S1 (P40682) Evalue: 2e-05	120	2	7.8	296	0.55	0.4	0.3	
contig21136:176:303:2 similar to V-type proton ATPase subunit E 1 (P11019) Evalue: 3e-05	80	2	52.4	42	1.54	2.91	2.91	
contig18185:1:284:3 similar to V-type proton ATPase subunit E 1 (P36543) Evalue: 1e-25	132	5 (3)	23.7	93	1.67	2.68	2.11	
contig20925:1:267:1 similar to V-type proton ATPase subunit E (P54611) Evalue: 2e-29	228	6 (4)	37.1	89	6.04	3.98	3.34	
contig08888:1:720:1 similar to V-type proton ATPase 116 kDa subunit a isoform 1 (Q5R422) Evalue: 7e-87	85	2	13.3	240	0.92	0.37	0.49	
contig08887:158:310:2 similar to V-type proton ATPase 116 kDa subunit a isoform 1 (Q8AVM5) Evalue: 7e-19	54	1	21.6	51	1.2			
contig08886:1:238:1 similar to Probable V-type proton ATPase 116 kDa subunit a (P30628) Evalue: 2e-28	49	1	19	79	0.74		0.74	
contig03390:223:1404:1 similar to V-type proton ATPase subunit C (Q9U5N1) Evalue: 8e-147	490	12 (10)	27.2	393	0.78	1.06	0.54	
contig08267:1:355:2 similar to V-type proton ATPase subunit e (Q20591) Evalue: 4e-19	36	1	6	117	0.5			
contig05261:1:326:3 similar to V-type proton ATPase subunit B, brain isoform (P62815) Evalue: 6e-41	201	5 (3)	34.6	107	1.13	1.79	2.69	
contig02085:242:1257:2 similar to V-type proton ATPase subunit H (Q9V3J1) Evalue: 2e-98	436	10 (8)	35.5	338	0.34	1.06	0.84	
contig00107:99:1058:3 similar to V-type proton ATPase subunit D 1 (Q9V7D2) Evalue: 2e-83	99	1	5	319	0.39	0.48	1.03	
contig26710:1:1127:3 similar to Obg-like ATPase 1 (Q7ZWM6) Evalue: 1e-155	601	12 (10)	37.2	374	2.89	1.71	1.71	
contig26232:65:1864:2 similar to ATPase family AAA domain-containing protein 3-A (Q58E76) Evalue: 0.0	67	2	2.5	600		0.1	0.27	
contig22225:72:1087:3 similar to ATPase ASNA1 homolog (Q5TRE7) Evalue: 4e-112	530	11 (9)	43.2	338	0.79	1.39	1.11	

## Supplementary

contig18662:1:441:1 similar to Vacuolar ATPase assembly integral membrane protein VMA21 (B8JLV7) Evalue: 3e-06	195	4 (2)	25.3	146	1.15		
contig16339:1:184:2 similar to Probable cation-transporting ATPase 13A1 (Q9EPE9) Evalue: 5e-19	47	1	20	60	1.08		
contig06158:1:2033:3 similar to Vesicle-fusing ATPase 2 (P54351) Evalue: 0.0	411	8	17.6	676		0.41	0.41
contig27544:274:1827:1 similar to Cytochrome P450 3A30 (Q9PVE8) Evalue: 5e-90	66	2	3.1	517		0.11	
contig27503:77:1735:2 similar to Cytochrome P450 2J6 (O54750) Evalue: 5e-80	104	2	5.3	552		0.16	
contig27115:1:426:1 similar to Cytochrome P450 2C25 (Q08078) Evalue: 2e-13	69	2	12.7	142		0.67	0.42
contig26568:95:361:2 similar to Cytochrome b-c1 complex subunit 10 (P07552) Evalue: 2e-05	139	6 (3)	31.8	88	2.6		
contig26329:1:486:1 similar to Cytochrome b5 (P00171) Evalue: 3e-22	191	4 (3)	31.1	161	1.14		
contig26196:1:647:2 similar to Cytochrome P450 2J6 (O54750) Evalue: 9e-10	127	2	14.4	215			0.29
contig24315:1:1220:3 similar to Cytochrome P450 2J6 (O54750) Evalue: 2e-74	64	2	3.5	405		0.15	0.23
contig24230:1:1077:1 similar to Cytochrome P450 2U1 (Q4V8D1) Evalue: 2e-66	79	1	3.9	358		0.16	
contig23923:1:1282:2 similar to Cytochrome P450 2B19 (O55071) Evalue: 5e-67	82	2	5.2	426		0.14	0.22
contig23746:198:2246:3 similar to NADPH--cytochrome P450 reductase (Q07994) Evalue: 0.0	249	5 (4)	9.5	682			0.18
contig23654:1:1510:2 similar to Cytochrome P450 2U1 (Q7Z449) Evalue: 7e-67	255	6	14.9	502		0.39	0.25
contig23646:1:1482:1 similar to Cytochrome P450 2J2 (P51589) Evalue: 1e-63	394	9 (8)	20.3	493		0.71	0.75
contig23353:1:440:3 similar to Cytochrome c oxidase subunit 6B (Q01519) Evalue: 2e-22	177	5	36.6	145	1.68		
contig23162:1:280:2 similar to Cytochrome b-c1 complex subunit 9 (Q8R111) Evalue: 2e-08	56	2	15.2	92	2.07	1.07	0.75
contig22050:1:1540:2 similar to Cytochrome P450 2C20 (P33262) Evalue: 2e-71	331	7 (6)	14.6	512		0.77	0.89
contig21472:1:1213:2 similar to Cytochrome P450 2U1 (Q4V8D1) Evalue: 5e-77	197	3	9.7	403		0.23	0.23
contig20178:83:1582:2 similar to Cytochrome P450 2B19 (O55071) Evalue: 3e-49	173	5	9.6	499		0.32	0.12
contig19895:1:358:2 similar to NADH-cytochrome b5 reductase 2 (Q0P487) Evalue: 2e-43	241	6 (5)	58.8	119		2.11	0.57
contig19354:1:1352:1 similar to Cytochrome P450 18a1 (Q95078) Evalue: 4e-37	464	11 (9)	28.9	450	0.58	1.15	0.65
contig18666:1:338:3 similar to Cytochrome c oxidase subunit 5A, mitochondrial (P55954) Evalue: 2e-27	168	3	46.8	111	1.32		
contig18322:1:728:3 similar to Cytochrome c oxidase subunit 4 isoform 2, mitochondrial (P80971) Evalue: 2e-21	400	18 (7)	48.1	241	1.73	4.01	3.26
contig17481:1:1652:3 similar to Cytochrome P450 3A24 (Q29496) Evalue: 1e-74	151	3	5.1	549		0.16	0.11
contig16613:76:1647:1 similar to Cytochrome P450 3A31 (O70537) Evalue: 3e-62	84	2	2.9	523		0.11	
contig15324:1:288:3 similar to Cytochrome b-c1 complex subunit 7 (P00129) Evalue: 8e-08	176	6 (5)	51.6	95	8.68		
contig11570:334:1468:1 similar to Cytochrome P450 2A9 (P24455) Evalue: 3e-36	147	3	8.5	378		0.4	0.16
contig09375:1:1074:1 similar to Cytochrome P450 2J2 (P51589) Evalue: 1e-56	136	3	9.8	357		0.26	
contig07909:1:449:3 similar to Cytochrome c oxidase subunit 6C (Q9CPQ1) Evalue: 7e-10	57	2	12.8	148	0.43		
contig07428:1:481:3 similar to Putative cytochrome P450 cyp-13B1 (O17624) Evalue: 6e-15	298	5 (4)	39.6	159		2.94	1.9
contig06063:154:1166:1 similar to Cytochrome P450 3A30 (Q9PVE8) Evalue: 3e-49	148	2	11.3	337		0.27	
contig05962:146:1027:2 similar to Cytochrome b5 domain-containing protein 2 (A2CES0) Evalue: 4e-39	88	4 (2)	6.5	293			0.21
contig02937:149:478:2 similar to Cytochrome c (Q6QLW4) Evalue: 8e-48	166	3	34.9	109	2.2		
contig02550:234:1586:3 similar to Cytochrome P450 2U1 (Q4V8D1) Evalue: 7e-74	250	4	13.8	450		0.28	0.2
contig02480:1:685:3 similar to Cytochrome c oxidase subunit 5B, mitochondrial (P10606) Evalue: 5e-15	189	6 (3)	19.8	227	0.88		
contig01924:1:1561:2 similar to Cytochrome b-c1 complex subunit 2, mitochondrial (P23004) Evalue: 7e-64	1084	41 (19)	50.5	519	1.99	4.85	4.7
contig01617:1:1058:3 similar to Cytochrome P450 2H1 (P05180) Evalue: 5e-53	147	4	12.3	351			0.37
contig01237:312:1775:3 similar to Cytochrome P450 2A9 (P24455) Evalue: 7e-49	174	4	8.8	487		0.26	
contig00880:106:1038:1 similar to Cytochrome c1, heme protein, mitochondrial (P00125) Evalue: 4e-61	435	18 (7)	33.9	310	1.01	2.33	2.21
contig00490:282:923:3 similar to Cytochrome b-c1 complex subunit Rieske, mitochondrial (Q69BJ7) Evalue: 3e-62	334	11 (6)	22.1	213	1.79	2.25	2.32

## Supplementary

contig00321:1:1219:2 similar to Cytochrome P450 3A4 (P08684) Evalue: 2e-68	504	13 (10)	34.8	405		3	2.72
contig23307:178:759:1 similar to GTP-binding protein SAR1 (Q23445) Evalue: 1e-74	530	15 (9)	57	193	4.67	4.45	3.73
contig26352:429:1127:3 similar to Rho-related GTP-binding protein RhoC (Q9PSX7) Evalue: 1e-99	324	6	37.9	232	2.01	1.17	0.94
contig26699:1:589:2 similar to Ras-like GTP-binding protein RYL1 (P41924) Evalue: 4e-19	33	1	3.1	195		0.28	
contig17610:1:1109:3 similar to Ras-related GTP-binding protein C (Q9HB90) Evalue: 6e-137	76	2	8.2	368	0.25	0.16	0.16
contig22397:1:1098:1 similar to Ras-related GTP-binding protein A (Q63486) Evalue: 6e-159	77	2	5.2	365		0.16	
contig02990:1:888:1 similar to Developmentally-regulated GTP-binding protein 2 (P55039) Evalue: 2e-132	133	3	12.2	295	0.2	0.54	0.46
contig05742:1:412:2 similar to Developmentally-regulated GTP-binding protein 1 (Q9Y295) Evalue: 5e-34	147	3	25.5	137	1.08		
contig12730:1:766:1 similar to Developmentally-regulated GTP-binding protein 1 (P43690) Evalue: 7e-110	81	2 (1)	5.5	255		0.33	
contig21323:1:561:1 similar to Developmentally-regulated GTP-binding protein 1 (Q9Y295) Evalue: 7e-82	238	4	26.2	187	1.06	0.78	1.4
contig20039:1580:1811:2 similar to GTP-binding protein 128up (P32234) Evalue: 8e-32	147	3 (2)	39.5	76	0.96		
contig19693:1:629:1 similar to GTP-binding protein 8 (B0S8I0) Evalue: 9e-22	101	2	11	209	0.3		
contig18824:1:222:1 similar to GTP-binding protein lepA (B1VY28) Evalue: 3e-05	26	1	8.2	73	0		
contig28286:1:1555:2 similar to Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial (P11181) Evalue: 7e-129	465	13 (10)	23.8	517	0.12	0.95	1.01
contig28056:1:780:1 similar to Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (Q07536) Evalue: 2e-116	638	14 (10)	59.8	259	1.22	3.77	4.96
contig27558:255:1303:3 similar to Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial (Q28479) Evalue: 1e-117	795	17 (13)	40.1	349	1.06	3.3	2.43
contig26946:46:1329:1 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial (P34943) Evalue: 1e-74	758	16 (13)	52	427	0.69	1.64	1.12
contig26279:25:684:1 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial (P91929) Evalue: 1e-29	124	2	10.5	220	0.27	0.27	0.83
contig26942:1:1185:1 similar to Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase (Q642M9) Evalue: 2e-57	595	14 (10)	30.7	394	1.85	1.6	1.59
contig26466:1:1569:1 similar to Aldehyde dehydrogenase family 8 member A1 (Q8BH00) Evalue: 7e-172	351	8	20.9	522		0.88	0.79
contig26049:1:355:1 similar to NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial (P23709) Evalue: 1e-52	154	4 (3)	29.7	118	0.78	1.15	2.19
contig25936:1:762:2 similar to Isocitrate dehydrogenase [NADP] cytoplasmic (Q5R9C5) Evalue: 1e-70	540	19 (12)	49.4	253	0.24	6.36	2.36
contig25872:119:1225:2 similar to Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (O44451) Evalue: 1e-135	570	16 (11)	39.4	368	0.48	2.12	2.37
contig25656:4:1527:1 similar to Proline dehydrogenase, mitochondrial (Q04499) Evalue: 2e-119	296	7	15.9	508		0.47	0.31
contig25594:1:255:2 similar to Malate dehydrogenase, mitochondrial (Q5NVR2) Evalue: 1e-30	191	6 (2)	66.7	84	2.7	6.51	4.28
contig25483:1682:2388:3 similar to Isocitrate dehydrogenase [NADP] cytoplasmic (Q9XSG3) Evalue: 8e-107	837	27 (14)	55.1	234	3.77	11.5	4.62
contig25474:1:581:3 similar to Estradiol 17-beta-dehydrogenase 12 (O57314) Evalue: 5e-35	101	2	15.6	192		0.5	
contig25396:1:1593:1 similar to Dihydropolypol dehydrogenase, mitochondrial (P09622) Evalue: 0.0	781	20 (15)	32.3	530	0.68	1.75	1.77
contig25322:103:1317:1 similar to Malate/L-sulfolactate dehydrogenase (P16142) Evalue: 3e-60	882	27 (18)	66.6	404	0.93	4.06	3.31
contig25109:1:2811:1 similar to Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial (Q4KLP0) Evalue: 0.0	431	10	16.6	936	0.13	0.35	0.26
contig25021:205:1170:1 similar to D-3-phosphoglycerate dehydrogenase (O43175) Evalue: 6e-99	644	19 (10)	36.8	321	4.57	3.2	1.91
contig24779:1:327:1 similar to 3-hydroxyisobutyrate dehydrogenase, mitochondrial (P31937) Evalue: 6e-38	273	4	64.8	108	0.66	1.76	0.66
contig24607:31:258:1 similar to Xanthine dehydrogenase (Q54FB7) Evalue: 3e-07	52	1	18.7	75		0.84	
contig24463:296:1435:2 similar to Glucose and ribitol dehydrogenase (Q5KTS5) Evalue: 1e-80	674	23 (10)	34	379	5.98	3.82	4.02
contig24413:1:621:1 similar to Dehydrogenase/reductase SDR family member 4 (Q8SPU8) Evalue: 9e-62	54	2	7.2	207		0.33	
contig24401:1:658:2 similar to Malate dehydrogenase, mitochondrial (Q5NVR2) Evalue: 6e-85	995	36 (14)	79.8	218	9.1	25.04	18.92
contig24248:1:1048:2 similar to Isocitrate dehydrogenase [NAD] subunit gamma, mitochondrial (P70404) Evalue: 9e-113	253	7 (6)	25.3	348	0.71	1.21	1.54

## Supplementary

contig24203:161:1945:2 similar to Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial (A7YWE4) Evalue: 0.0	408	7	16.3	594		0.45	0.47
contig23953:1:1637:3 similar to Aldehyde dehydrogenase X, mitochondrial (P30837) Evalue: 0.0	1120	38 (19)	40.6	544	1.18	2.88	3.11
contig23852:1:2534:3 similar to Aldehyde dehydrogenase family 16 member A1 (A6QR56) Evalue: 8e-58	249	5	8.9	843		0.19	
contig23849:1:530:1 similar to Malate dehydrogenase, mitochondrial (Q32LG3) Evalue: 5e-46	114	2 (1)	10.8	176	0.36	0.9	0.58
contig23804:63:702:3 similar to Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial (P00348) Evalue: 1e-59	347	6 (5)	30	213	0.73	1.14	1.62
contig23626:1:835:2 similar to Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (Q27928) Evalue: 1e-77	1057	25 (15)	70	277	5.46	6.62	6
contig23295:95:1060:2 similar to Pyruvate dehydrogenase protein X component, mitochondrial (Q8BKZ9) Evalue: 3e-19	199	4	17.8	321		0.43	0.43
contig23104:1:464:3 similar to Fatty aldehyde dehydrogenase (P30839) Evalue: 5e-31	132	2	14.4	153	0.45	0.65	0.45
contig23034:209:1171:2 similar to 3-oxo-5-alpha-steroid 4-dehydrogenase 2 (P31213) Evalue: 1e-26	113	4 (3)	8.4	320	0.47	0.3	0.3
contig22977:68:553:2 similar to Probable NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12 (Q9N2W7) Evalue: 3e-32	83	2	10.6	161	0.64	0.39	0.39
contig22763:1:875:2 similar to L-2-hydroxyglutarate dehydrogenase, mitochondrial (Q9N4Z0) Evalue: 3e-73	229	4	17.5	291		0.48	0.48
contig22266:1:2016:1 similar to 2-oxoglutarate dehydrogenase E1 component-like, mitochondrial (Q9ULD0) Evalue: 0.0	558	13 (12)	25	671		1.04	0.76
contig21957:311:2059:2 similar to Glucose dehydrogenase [acceptor] (P18172) Evalue: 2e-60	141	3	6.2	583	0.16		
contig21923:132:1186:3 similar to Hydroxysteroid dehydrogenase-like protein 2 (Q66KC4) Evalue: 1e-104	502	13 (9)	32.8	351	1.92	2.23	2.15
contig21715:1:127:1 similar to Alcohol dehydrogenase class-3 (Q54TC2) Evalue: 2e-08	87	1	28.6	42	1.62	1.62	1.62
contig21579:1:845:1 similar to Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase (Q6DF30) Evalue: 4e-58	247	5 (4)	16.4	281	0.83	0.5	0.5
contig21261:1:794:2 similar to Estradiol 17-beta-dehydrogenase 12 (O57314) Evalue: 9e-43	255	6	26.1	264	0.89		0.24
contig21168:1:308:3 similar to 10-formyltetrahydrofolate dehydrogenase (Q6GNL7) Evalue: 9e-41	72	2	20.6	102		0.69	
contig21097:128:1681:2 similar to Glucose-6-phosphate 1-dehydrogenase (Q29492) Evalue: 4e-160	868	20 (17)	40.8	517	0.89	1.71	1.34
contig20967:116:1264:2 similar to Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase (Q642M9) Evalue: 5e-60	405	13 (9)	30.6	382	0.45	1.78	1.19
contig20526:1:608:3 similar to Malate dehydrogenase, cytoplasmic (O48905) Evalue: 3e-39	621	27 (10)	49.3	201	4.03	10.05	9.28
contig20357:221:1750:2 similar to Inosine-5--monophosphate dehydrogenase 2 (Q3SWY3) Evalue: 0.0	680	14 (11)	31.4	509	1.06	2.18	1.38
contig20194:512:900:3 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6 (Q0MQA3) Evalue: 4e-22	134	3	26.6	128	0.82		
contig20136:95:615:2 similar to Probable methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (Q17M80) Evalue: 2e-57	378	10 (7)	41.6	173	1.69	4.36	3.95
contig20081:1:667:3 similar to NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10 (Q1HPL8) Evalue: 2e-33	40	1	6.8	221			0.24
contig20073:1377:1828:3 similar to Dehydrogenase/reductase SDR family member 11 (Q3U0B3) Evalue: 3e-14	285	8 (7)	66.4	149	1.16	3.66	1.83
contig20063:1:613:2 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 (Q9DCJ5) Evalue: 5e-32	226	6 (4)	27.6	203		0.92	0.92
contig19828:1:1197:1 similar to Alcohol dehydrogenase class-3 (P81431) Evalue: 2e-146	260	5	14.8	398	1.09	0.93	0.66
contig19788:1:833:3 similar to NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial (P0CB83) Evalue: 4e-80	115	4 (3)	14.5	276		0.61	0.61
contig19760:1:366:1 similar to Inactive hydroxysteroid dehydrogenase-like protein 1 (Q3SXM5) Evalue: 8e-18	66	2	17.2	122		0.58	
contig19370:162:1034:3 similar to Estradiol 17-beta-dehydrogenase 12 (Q28IU1) Evalue: 6e-42	234	7 (6)	27.1	291	0.88	1.38	1.4
contig19336:176:484:2 similar to L-lactate dehydrogenase A chain (P04642) Evalue: 2e-18	259	6 (4)	36.9	103	2.71	2.29	2.29
contig19280:1:414:1 similar to 3-hydroxyisobutyrate dehydrogenase, mitochondrial (Q99L13) Evalue: 1e-41	80	2	21	138			0.52
contig19122:2343:3388:3 similar to Probable 10-formyltetrahydrofolate dehydrogenase ALDH1L2 (Q3SY69) Evalue: 5e-155	325	8 (7)	21.6	347		1.05	0.6
contig19105:36:1151:3 similar to 2-oxoisovalerate dehydrogenase subunit beta, mitochondrial (P21953) Evalue: 2e-130	397	8 (7)	28.6	371	0.26	0.72	1.17
contig19099:1:236:3 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial (P91929) Evalue: 9e-14	128	3 (2)	44.9	78			0.9
contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	1738	70 (28)	58.5	593	4.85	17.19	15.05
contig18317:1:198:1 similar to Dehydrogenase/reductase SDR family member 4 (Q5RCF8) Evalue: 9e-10	96	2	66.2	65		1.23	

## Supplementary

contig18256:135:2303:3 similar to Glycerol-3-phosphate dehydrogenase, mitochondrial (Q4R755) Evalue: 0.0	653	12 (11)	19.5	722		0.6	0.67
contig18220:95:1021:2 similar to Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial (P21914) Evalue: 1e-111	374	10 (7)	28.6	308	0.56	1.67	1.99
contig18205:1:513:1 similar to Alpha-aminoadipic semialdehyde dehydrogenase (Q9DBF1) Evalue: 1e-70	136	5 (3)	22.9	170		0.94	0.65
contig18143:1:1164:1 similar to Sorbitol dehydrogenase (Q4R639) Evalue: 2e-118	555	15 (11)	38.2	387	1.34	2.54	2.02
contig17984:1:986:2 similar to Estradiol 17-beta-dehydrogenase 12-A (Q6P3L6) Evalue: 4e-58	191	5 (4)	14.9	328		1.04	0.5
contig17746:1:1516:2 similar to 6-phosphogluconate dehydrogenase, decarboxylating (P41570) Evalue: 0.0	1062	30 (17)	45.2	504	4.6	4.23	3.76
contig17727:6:713:3 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 11 (Q9D8B4) Evalue: 2e-04	140	3	20.9	235		0.42	0.26
contig17689:166:762:1 similar to Dehydrogenase/reductase SDR family member 11 (Q71R50) Evalue: 4e-34	108	3 (2)	12.1	199		0.32	0.76
contig17536:1:460:2 similar to Probable NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial (Q19724) Evalue: 6e-20	159	3	32.2	152	0.92		
contig17524:1:1401:1 similar to Probable saccharopine dehydrogenase (Q8NBX0) Evalue: 3e-91	336	7	20.6	466	0.36	0.54	0.45
contig17480:61:1323:1 similar to Epidermal retinol dehydrogenase 2 (Q7TQA3) Evalue: 5e-62	512	15 (12)	35	420		2.35	0.92
contig13474:1:938:1 similar to Xanthine dehydrogenase (P10351) Evalue: 3e-45	27	1	3.2	312	0.18		
contig13473:1:383:3 similar to Xanthine dehydrogenase/oxidase (P22985) Evalue: 5e-20	86	2	17.5	126	0.54		
contig13155:1:596:3 similar to Short chain dehydrogenase/reductase family 9C member 7 (A4IFM3) Evalue: 1e-26	210	4	27.4	197		1.06	0.45
contig11886:1:608:3 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4 (Q6PBH5) Evalue: 5e-08	180	5 (4)	26.4	201	1.22		
contig11075:81:1535:3 similar to Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial (Q9D2G2) Evalue: 6e-109	385	11 (8)	19.8	484	0.86	1.56	1.31
contig09642:410:2035:2 similar to Succinate-semialdehyde dehydrogenase, mitochondrial (P51650) Evalue: 5e-140	570	10 (9)	21.8	541	0.18	0.72	0.78
contig09557:1:921:1 similar to Probable 10-formyltetrahydrofolate dehydrogenase ALDH1L2 (Q3SY69) Evalue: 6e-74	450	7	35.8	307	0.2	1.41	0.89
contig09413:1:212:3 similar to Hydroxysteroid dehydrogenase-like protein 2 (Q66KC4) Evalue: 2e-09	138	2	30.4	69	1.2	1.2	
contig09337:1:283:2 similar to Probable methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (P52713) Evalue: 5e-38	32	1	7.4	94		0.66	1.1
contig09040:1791:2687:1 similar to Retinol dehydrogenase 13 (Q8CEE7) Evalue: 6e-41	235	6	24.5	298		0.61	0.46
contig08973:1:867:2 similar to Dehydrogenase/reductase SDR family member 1 (Q99L04) Evalue: 7e-80	693	16 (11)	54.2	288	6.41	5.43	3.24
contig08535:1:521:3 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13 (Q95KV7) Evalue: 7e-17	110	2	14	172	0.51		
contig07806:113:1741:2 similar to Xanthine dehydrogenase (Q54FB7) Evalue: 5e-91	853	23 (18)	33	542	1.44	2.6	1.71
contig07602:172:402:1 similar to NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial (P42028) Evalue: 4e-06	82	2	23.4	77	0.84	1	1
contig07118:1:1064:3 similar to Probable isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial (Q9VWH4) Evalue: 3e-159	952	29 (16)	58.6	353	2.47	6.04	6.34
contig07064:139:386:1 similar to Glucose and ribitol dehydrogenase (Q5KTS5) Evalue: 1e-10	191	5 (4)	57.3	82	5.54	4.48	3.71
contig06986:298:1467:1 similar to Probable pyruvate dehydrogenase E1 component subunit alpha, mitochondrial (P52899) Evalue: 7e-140	768	18 (14)	39.1	389	0.34	1.92	1.91
contig06815:1:865:2 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3 (Q9BU61) Evalue: 2e-25	68	2	6.3	287		0.21	0.33
contig06046:155:1205:2 similar to Aldehyde dehydrogenase family 3 member B1 (Q5XI42) Evalue: 4e-95	379	7	25.7	350	0.27	1.13	1.07
contig05917:78:1079:3 similar to Estradiol 17-beta-dehydrogenase 12 (Q4R5G7) Evalue: 7e-64	124	3	10.5	333		0.38	1.09
contig05810:75:977:3 similar to 3-oxo-5-alpha-steroid 4-dehydrogenase 1 (P24008) Evalue: 4e-63	67	2	6.7	300	0.65	0.2	
contig05781:891:1874:3 similar to Dihydropyrimidine dehydrogenase [NADP+] (Q6NYG8) Evalue: 1e-158	275	4	18.9	328		0.44	0.2
contig05781:1:729:1 similar to Dihydropyrimidine dehydrogenase [NADP+] (Q55FT1) Evalue: 3e-84	208	5 (4)	25.2	242		1.3	0.66
contig05654:1:470:3 similar to L-2-hydroxyglutarate dehydrogenase, mitochondrial (A7SMW7) Evalue: 3e-45	212	4 (3)	27.7	155		1.06	
contig05570:1:1084:2 similar to 3-oxo-5-beta-steroid 4-dehydrogenase (P51857) Evalue: 6e-68	460	15 (8)	27.2	360		1.24	1.51

## Supplementary

contig05185:1:1054:2 similar to Alcohol dehydrogenase class-3 (P79896) Evalue: 1e-105	485	11 (9)	36.9	350	1.96	1.28	0.69
contig05086:1:335:3 similar to Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (O57656) Evalue: 6e-17	350	11 (5)	49.5	111	4.86	6.25	4.08
contig04930:197:1183:2 similar to Estradiol 17-beta-dehydrogenase 12-B (Q8AVY8) Evalue: 3e-66	684	15 (12)	47.6	328	0.36	2.29	1.36
contig04807:1:472:2 similar to Dehydrogenase/reductase SDR family member 11 (Q3ZBV9) Evalue: 1e-20	60	1	10.8	157	0.43	0.38	0.38
contig03952:1:312:2 similar to 2-oxoglutarate dehydrogenase E1 component, mitochondrial (Q02218) Evalue: 2e-37	78	3 (2)	20.4	103		2.12	0.67
contig03914:1:262:2 similar to Probable 3-hydroxyisobutyrate dehydrogenase, mitochondrial (Q54CX6) Evalue: 3e-12	141	3 (2)	40.2	87		0.87	0.87
contig03543:125:1543:2 similar to NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial (Q91WD5) Evalue: 0.0	424	13 (9)	22	472	0.42	0.79	1.01
contig03403:1:1416:3 similar to Dihydropolyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial (Q8BMF4) Evalue: 6e-108	456	10 (9)	26.3	471	0.76	0.88	1.27
contig03161:1:931:2 similar to 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial (A5A6H9) Evalue: 5e-120	491	9	43.4	309		1.26	0.44
contig03158:1:611:1 similar to 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial (P11960) Evalue: 1e-25	126	3	21.2	203		0.5	0.64
contig02601:1:511:2 similar to NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 (Q4R5J1) Evalue: 1e-28	94	2	12.4	169	0.56		
contig02251:1:518:3 similar to L-lactate dehydrogenase (Q95028) Evalue: 8e-61	334	8 (6)	43.3	171	1.83	4.21	3.5
contig02052:156:636:3 similar to Malate dehydrogenase, cytoplasmic (P40925) Evalue: 5e-56	413	14 (6)	55.6	160	2.24	7.39	4.38
contig01310:1889:2851:3 similar to Putative L-aspartate dehydrogenase (Q5FW48) Evalue: 1e-70	174	3	15.9	320		0.31	0.43
contig00798:1:1071:1 similar to Alpha-aminoacidic semialdehyde dehydrogenase (P49419) Evalue: 4e-131	396	13 (8)	26.1	357	0.77	1.52	1.34
contig00412:206:1279:2 similar to Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating (Q9R1J0) Evalue: 5e-106	70	2	5.6	357		0.17	
contig00403:1:1267:1 similar to Alcohol dehydrogenase class-3 (P46415) Evalue: 5e-96	267	7 (6)	21.1	422		0.81	0.37
contig05626:1:458:3 similar to Succinate dehydrogenase cytochrome b560 subunit, mitochondrial (P70097) Evalue: 1e-27	34	1	5.3	151		0.4	
contig05626:1:458:3 similar to Succinate dehydrogenase cytochrome b560 subunit, mitochondrial (P70097) Evalue: 1e-27	101	2	17.9	151	0.44		0.6
contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	1520	96 (23)	62.5	416	11.27	23.08	26.61
contig26424:9:1010:3 similar to Glyceraldehyde-3-phosphate dehydrogenase (Q9UR38) Evalue: 2e-124	67	4 (2)	4.5	333		0.19	
contig01804:1:688:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (Q75712) Evalue: 4e-70	82	4 (2)	8.3	228		0.46	
contig28291:1:287:3 similar to Short-chain specific acyl-CoA dehydrogenase, mitochondrial (Q3ZBF6) Evalue: 1e-37	105	2	23.4	94	0.77		0.77
contig28031:1:173:2 similar to Short-chain specific acyl-CoA dehydrogenase, mitochondrial (Q07417) Evalue: 6e-19	124	2	38.6	57	2.21	3.2	3.2
contig27778:1:335:3 similar to 3-hydroxyacyl-CoA dehydrogenase type-2 (O08756) Evalue: 5e-29	196	6 (4)	43.6	110		4.39	2.42
contig26482:1:1206:1 similar to Medium-chain specific acyl-CoA dehydrogenase, mitochondrial (P41367) Evalue: 6e-150	586	14 (11)	37.7	401	1.6	1.9	2.38
contig25445:81:384:3 similar to Short-chain specific acyl-CoA dehydrogenase, mitochondrial (Q3ZBF6) Evalue: 5e-17	170	3	47.5	101			1.19
contig22989:1:435:1 similar to 3-hydroxyacyl-CoA dehydrogenase type-2 (O18404) Evalue: 6e-42	230	8 (5)	45.1	144	4.78	2.21	2.21
contig22215:21:483:3 similar to 3-hydroxyacyl-CoA dehydrogenase type-2 (O18404) Evalue: 1e-23	262	5 (3)	30.5	154		2.63	1.99
contig19834:117:958:3 similar to Short-chain specific acyl-CoA dehydrogenase, mitochondrial (P15651) Evalue: 8e-86	153	4 (2)	10.7	280	0.23	0.37	0.68
contig17123:1:404:3 similar to 3-hydroxyacyl-CoA dehydrogenase type-2 (O18404) Evalue: 1e-28	185	5 (3)	33.6	134	1.36	1.08	1.28
contig06029:196:2172:1 similar to Very long-chain specific acyl-CoA dehydrogenase, mitochondrial (P50544) Evalue: 0.0	1219	33 (22)	44.4	658	1.02	2.46	2.57
contig04564:1:305:3 similar to Probable 3-hydroxyacyl-CoA dehydrogenase F54C8.1 (P34439) Evalue: 4e-30	263	8 (5)	74	100	2.29	2.92	2.29
contig01718:85:1824:1 similar to Acyl-CoA dehydrogenase family member 9, mitochondrial (Q9H845) Evalue: 5e-64	277	5	10.7	579	0.1	0.28	0.16
contig00517:206:1202:2 similar to Probable acyl coa dehydrogenase 6 (P34275) Evalue: 6e-74	97	3	14.2	332		0.29	
contig25839:22:1331:1 similar to Glutaryl-CoA dehydrogenase, mitochondrial (Q8HXX8) Evalue: 3e-157	239	10 (5)	14.9	436	0.22	0.39	0.63
contig22870:100:1383:1 similar to Isovaleryl-CoA dehydrogenase, mitochondrial (P12007) Evalue: 9e-170	501	9 (8)	29.7	427	0.14	0.82	0.82
contig00282:1:1286:3 similar to Isobutyryl-CoA dehydrogenase, mitochondrial (Q9UKU7) Evalue: 3e-159	265	5	14.8	427	0.22	0.71	0.53
contig28269:30:998:3 similar to Splicing factor, arginine/serine-rich 1 (Q5ZML3) Evalue: 3e-45	229	8 (6)	23.6	322	1.59	0.97	0.87
contig16592:1:1150:2 similar to Probable splicing factor ECU05_1440 (Q8SRU2) Evalue: 5e-04	84	2	5.2	382	0.25	0.16	0.35

Supplementary

contig09104:39:1472:3 similar to Splicing factor U2AF 50 kDa subunit (Q24562) Evalue: 2e-150	98	2	5.5	477	0.18		
contig04334:1:848:3 similar to Splicing factor, arginine/serine-rich 2 (Q6PDU1) Evalue: 2e-26	141	5 (3)	11.7	281	0.34		
contig03174:93:776:3 similar to Splicing factor 3A subunit 2 (Q15428) Evalue: 5e-93	48	1	6.2	227	0.26		
contig03174:93:776:3 similar to Splicing factor 3A subunit 2 (Q15428) Evalue: 5e-93	86	2	9.7	227			0.13
contig18788:35:1345:2 similar to Splicing factor 3B subunit 1 (O57683) Evalue: 0.0	157	3	8.3	436	0.28		
contig17573:1:1042:2 similar to Pre-mRNA-splicing factor SYF1 (Q9HCS7) Evalue: 4e-110	87	2	5.8	347	0.08		
contig27354:1:1788:3 similar to Splicing factor 3 subunit 1 (Q8K4Z5) Evalue: 4e-106	107	2	6.4	595	0.1		
contig26908:1:1585:2 similar to Pre-mRNA-processing-splicing factor 8 (Q6P2Q9) Evalue: 0.0	126	4	7.6	527	0.33		
contig26461:200:1774:2 similar to Splicing factor, proline- and glutamine-rich (Q8VIJ6) Evalue: 7e-64	112	3	6.7	524	0.18		
contig00644:1:1105:2 similar to Splicing factor 45 (Q96I25) Evalue: 1e-38	30	1	1.6	367		0.16	
similar to Splicing factor 45 (Q96I25) Evalue: 1e-38	29	2 (1)	1.6	367	0		0
contig17532:183:563:3 similar to Transcription elongation factor B polypeptide 2 (P62870) Evalue: 4e-15	237	3	33.3	126	1.16		
contig24493:168:1133:3 similar to Transcription elongation factor A protein 2 (Q15560) Evalue: 2e-71	241	4	21.8	321	0.42		
contig21456:1:519:1 similar to Transcription elongation factor B polypeptide 1 (P83940) Evalue: 5e-36	168	5 (4)	22.7	172	1.22		



### 7.3 Supplementary III

**Identification of differentially regulated proteins in active *versus* tun state as determined by DIGE experiment.** Generated MS/MS data were searched against the 454 tardigrade protein database. Spot number, protein annotation, accession number, total protein score, protein mass, protein pI, number of matched peptides, sequence coverage, Student's T test values and average ratio values are listed. A positive average ratio value indicates up-regulation in tun state and negative average ratio value up-regulation in active state.

Spot no	Protein description	Protein score	Protein mass	Protein matches (significant unique peptides)	Protein coverage (%)	Protein pl	T-test	Av. Ratio
1	contig03976:126:2186:3 similar to Chaperone protein dnaK (A6T4F4) Evalue: 0.0	291	75009	5 (5)	10,1	6,02	7,80E-005	1,52
2	Not detectable on stained gel						1,80E-005	1,48
3	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	93	17856	2 (2)	16,9	6,4	7,80E-006	1,48
4	Not detectable on stained gel						4,50E-005	1,44
5	contig18537:1:1312:2 No Annotation	53	49479	1 (1)	3,7	5,84	0,00071	1,44
6	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	164	17856	3 (3)	24,1	6,4	2,70E-005	1,42
7	contig18537:1:1312:2 No Annotation	58	49479	1 (1)	3,7	5,84	0,00042	1,41
8	contig01844:1:445:1 No Annotation	100	15778	2 (1)	10,1	6,05	1,80E-007	1,39
9	contig01844:1:445:1 No Annotation	122	15778	2 (1)	10,1	6,05	7,20E-007	1,37
	contig26098:1:627:1 similar to Glutathione S-transferase omega-1 (P78417) Evalue: 6e-39	41	24260	1 (1)	4,8	8,58		
	contig24910:1:199:2 No Annotation	38	7556	1 (1)	12,3	4,9		
10	contig08235:860:1596:2 No Annotation	370	27856	6 (6)	34,7	5,65	4,70E-005	1,35
	contig08235:1:820:2 No Annotation	123	30752	2 (2)	8,5	8,66		
	contig24910:1:199:2 No Annotation	38	7556	1 (1)	12,3	4,9		
11	No significant hits						0,0012	1,35
12	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	125	64906	3 (3)	5,6	5,47	0,0051	1,35
	contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	54	14492	1 (1)	9,4	4,82		
	contig24910:1:199:2 No Annotation	30	7556	1 (1)	12,3	4,9		
13	contig20526:1:608:3 similar to Malate dehydrogenase, cytoplasmic (O48905) Evalue: 3e-39	134	22687	3 (3)	13,4	6,13	1,10E-005	1,34
	contig02052:156:636:3 similar to Malate dehydrogenase, cytoplasmic (P40925) Evalue: 5e-56	60	17605	1 (1)	6,9	8,52		
	contig24910:1:199:2 No Annotation	30	7556	1 (1)	12,3	4,9		
14	contig20526:1:608:3 similar to Malate dehydrogenase, cytoplasmic (O48905) Evalue: 3e-39	109	22687	2 (1)	12,9	6,13	0,0026	1,33
	contig28264:1:1252:2 similar to Glyceraldehyde-3-phosphate dehydrogenase (P17244) Evalue: 8e-134	41	46596	1 (1)	3,6	7,71		
	contig23626:1:835:2 similar to Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (Q27928) Evalue: 1e-77	37	30745	1 (1)	3,6	6,12		
	contig24910:1:199:2 No Annotation	36	7556	1 (1)	12,3	4,9		
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	36	17856	1 (1)	7,8	6,4		
15	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	227	22292	4 (4)	24	5,26	0,0038	1,31
	contig18449:1:1229:3 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 2e-87	165	44803	3 (3)	7,8	8,14		
	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	66	65433	1 (1)	1,9	8,62		
16	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	813	64906	15 (15)	30,2	5,47	0,0015	1,31
	contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	187	14492	3 (3)	27,3	4,82		
17	contig10939:1:797:2 No Annotation	143	29117	3 (3)	18,1	6,96	3,90E-007	1,3
18	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	132	41570	3 (3)	7,7	6,6	0,00027	1,3
19	contig05967:88:688:1 similar to Putative aminopeptidase W07G4.4 (Q27245) Evalue: 9e-13	27	22292	1 (1)	9	5,26	6,20E-005	1,3
20	contig03109:97:1837:1 similar to Lamin Dm0 (P08928) Evalue: 4e-60	63	66260	1 (1)	1,7	5,33	5,20E-005	1,3
	contig18537:1:1312:2 No Annotation	46	49479	1 (1)	3,7	5,84		

## Supplementary

21	contig03976:126:2186:3 similar to Chaperone protein dnaK (A6T4F4) Evalue: 0.0	421	75009	8 (8)	14,1	6,02	0,00019	1,3
	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	46	56002	1 (1)	2,4	6,01		
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	33	35963	1 (1)	3,2	9,92		
22	contig22876:95:450:2 similar to Actin, cytoplasmic 1 (P68142) Evalue: 3e-67	58	13132	1 (1)	9,3	5,46	0,00024	1,3
23	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	103	64906	2 (2)	4,7	5,47	1,20E-005	1,3
	contig25323:751:1956:1 similar to Basement membrane-specific heparan sulfate proteoglycan core protein (P98160) Evalue: 2e-15	86	44588	2 (2)	5	8,68		
	contig05208:1:597:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 3e-50	34	22851	1 (1)	7,5	5,37		
24	No significant hits						0,00027	1,29
25	No significant hits						0,00073	1,28
26	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	185	41570	5 (5)	12,4	6,6	0,0044	1,27
27	contig06373:1:1480:1 similar to Vitellogenin-4 (P18947) Evalue: 9e-11	56	56002	1 (1)	2,4	6,01	4,70E-005	1,27
	contig04531:1:1454:1 similar to Apolipoporphins (Q9U943) Evalue: 7e-11	46	55650	1 (1)	2,3	6,86		
	contig18438:8:349:2 No Annotation	35	12958	1 (1)	9,7	8,58		
28	No significant hits						0,00088	1,27
29	No significant hits						2,80E-005	1,27
30	contig21929:197:901:2 No Annotation	474	23354	7 (6)	34,5	6,38	3,20E-006	1,26
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	393	17856	7 (6)	50,6	6,4		
	contig05847:1:205:2 No Annotation	89	6597	2 (2)	65,7	8,42		
31	contig24956:1:847:3 similar to Phosphoglycerate kinase (P51903) Evalue: 2e-108	335	30960	6 (6)	26,7	5,85	0,015	1,25
	contig18673:1:499:2 similar to Major egg antigen (P12812) Evalue: 2e-05	67	17856	1 (1)	9	6,4		
	contig18362:1:1784:3 similar to Glutamate dehydrogenase 1, mitochondrial (P00367) Evalue: 0.0	55	65433	1 (1)	1,9	8,62		
	contig24910:1:199:2 No Annotation	35	7556	1 (1)	12,3	4,9		
32	contig00103:97:441:1 similar to Protein disulfide-isomerase A6 (Q63081) Evalue: 9e-26	64	12685	1 (1)	12,3	7,68	0,0022	1,25
	contig00023:136:1154:1 No Annotation	58	37758	1 (1)	2,9	5,95		
	contig00443:1:204:1 No Annotation	57	7230	1 (1)	20,9	9,3		
33	contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	237	14492	4 (4)	39,8	4,82	0,031	1,25
	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	104	64906	2 (2)	4	5,47		
	contig04581:1:1472:3 similar to Tubulin alpha-4A chain (P68368) Evalue: 0.0	82	55209	2 (2)	5,7	5,25		
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	68	36658	2 (2)	6	5,84		
34	No significant hits						3,50E-005	1,25
35	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	951	64906	17 (17)	34,2	5,47	0,0082	1,25
	contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	224	14492	4 (4)	39,8	4,82		
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	30	36658	1 (1)	3,1	5,84		
36	contig24527:81:472:3 similar to Fructose-bisphosphate aldolase C (P53448) Evalue: 7e-46	127	14046	2 (2)	20,8	5,84	0,012	-1,25
37	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	39	29873	2 (1)	3,9	5,36	0,0031	-1,25
38	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	173	35963	4 (4)	12,6	9,92	2,00E-006	-1,26
39	contig01298:1:2065:3 similar to Catalase HPII (P21179) Evalue: 0.0	90	76798	2 (2)	3,2	5,98	0,0077	-1,27
40	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	118	29873	3 (3)	13,7	5,36	0,0049	-1,27
	contig26256:1:544:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-37	65	20475	1 (1)	6,6	5,13		
	contig08625:1:110:2 No Annotation	36	4172	1 (1)	22,2	9,05		

## Supplementary

	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	29	36658	1 (1)	3,1	5,84		
41	contig24758:1:1673:3 similar to Paramyosin (Q86RN8) Evalue: 2e-126	122	64906	3 (3)	5,6	5,47	7,00E-007	-1,29
	contig02794:61:444:1 similar to Paramyosin (Q86RN8) Evalue: 3e-32	49	14492	1 (1)	9,4	4,82		
	contig26256:1:544:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-37	37	20475	1 (1)	6,6	5,13		
	contig02293:1:648:1 similar to Vitellogenin-6 (P18948) Evalue: 5e-14	33	24857	1 (1)	4,2	4,86		
	contig15494:1:257:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 8e-18	30	9528	1 (1)	12,9	4,83		
42	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	202	29873	5 (5)	17,6	5,36	0,00052	-1,29
	contig24360:1:563:3 No Annotation	176	21499	3 (3)	21,4	5		
	contig00124:1:1376:3 similar to Eukaryotic initiation factor 4A-II (Q8JFP1) Evalue: 2e-166	114	52397	2 (2)	4,6	5,75		
	contig26443:1:303:1 No Annotation	75	11655	1 (1)	12,9	4,72		
	contig26256:1:544:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-37	71	20475	1 (1)	6,6	5,13		
	contig00982:1:961:2 similar to Actin-5C (P10987) Evalue: 3e-137	50	36658	1 (1)	3,4	5,84		
	contig08625:1:110:2 No Annotation	38	4172	1 (1)	22,2	9,05		
	contig24910:1:199:2 No Annotation	32	7556	1 (1)	12,3	4,9		
	contig02295:455:757:2 No Annotation	31	11684	1 (1)	21,8	6,82		
43	No significant hits						0,00025	-1,3
44	No significant hits						0,0001	-1,3
45	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	29	50376	1 (1)	1,8	8,51	0,00026	-1,34
46	No significant hits						4,60E-006	-1,36
47	No significant hits						6,80E-005	-1,36
48	contig23314:1:731:3 similar to Protein I(2)37Cc (P24156) Evalue: 2e-102	295	27164	6 (5)	22,3	5,84	0,00024	-1,37
	contig06595:1:952:2 similar to Vitellogenin-1 (P87498) Evalue: 3e-07	30	35963	1 (1)	3,2	9,92		
49	contig20766:1:786:1 similar to Uncharacterized oxidoreductase yhdF (O07575) Evalue: 1e-49	244	27880	4 (4)	19,2	6,92	0,043	-1,38
	contig01625:1:1375:2 similar to Elongation factor 1-alpha (P41752) Evalue: 0.0	79	50376	2 (2)	4,2	8,51		
	contig05892:363:620:3 similar to Uncharacterized oxidoreductase yhdF (O07575) Evalue: 2e-10	31	9137	1 (1)	15,1	9,05		
50	contig25886:1:136:2 No Annotation	34	5205	1 (1)	24,4	4,46	0,002	-1,4
51	No significant hits						0,00093	-1,4
52	contig25886:1:136:2 No Annotation	28	5205	1 (1)	24,4	4,46	3,50E-005	-1,4
53	No significant hits						0,015	-1,41
54	No significant hits						0,0012	-1,41
55	contig24586:1:771:2 similar to Vitellogenin-6 (P18948) Evalue: 5e-10	151	29873	4 (4)	17,2	5,36	0,053	-1,52
	contig24360:1:563:3 No Annotation	120	21499	2 (2)	13,9	5		
	contig26256:1:544:1 similar to Myosin heavy chain, muscle (P05661) Evalue: 4e-37	89	20475	2 (2)	11	5,13		
	contig26443:1:303:1 No Annotation	70	11655	1 (1)	12,9	4,72		
	contig24531:1:590:3 No Annotation	54	22259	1 (1)	7,1	4,65		
	contig08625:1:110:2 No Annotation	42	4172	1 (1)	47,2	9,05		
	contig00641:1:549:1 similar to Myosin heavy chain, striated muscle (P24733) Evalue: 3e-50	31	21089	1 (1)	6	7,88		
56	contig24910:1:199:2 No Annotation	26	7556	1 (0)	12,3	4,9	1,20E-005	-1,53
57	contig20019:1:1092:1 similar to Arginine kinase (Q95V58) Evalue: 1e-145	89	41570	2 (2)	5,8	6,6	0,0047	-1,59
	contig01971:1:138:399:3 No Annotation	62	9637	2 (2)	29,9	5,8		

## 8 Curriculum Vitae

### Personal Data

Name Elham Schokraie

Date/place of Birth 24.02.1980 in Teheran, Iran

Address Goethestr. 2  
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### Education

Feb. `07- Jul. `10 Ph. D. in functional proteomics  
“Functional Analysis of Dynamic Processes in *Milnesium tardigradum*”  
Functional Proteome Analysis (Dr. Schnölzer)  
German Cancer Research Center, Germany  
<http://www.dkfz.de/index.html>  
Proteomics group of FUNCRYPTA project  
<http://www.funcrypta.de/www/en/>

Apr. `06- Sep. `06 Diploma research work  
“Studies on mitochondrial respiratory chain complexes and  
supercomplexes from bovine heart”  
Physical Biochemistry Research group (Prof. Dr. N. Dencher)  
<http://www.biochemie.tu-darmstadt.de/Dencher/>  
Technical University of Darmstadt, Germany

Sep. `04- Jun. `05 Erasmus exchange program  
University of Bristol, England  
<http://www.bristol.ac.uk/>

Okt. `00- Sep. `06 Diploma Degree Chemistry  
Technical University of Darmstadt, Germany

Aug. `97- Jun. `00 High School (A level), Darmstadt, Germany

Aug. `96- Jun. `97 Secondary School, Groß Gerau, Germany

Aug. `87-Jan. `96 Primary and High School (A level), Teheran, Iran

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Dossenheim, den 15.07.2010

### Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich meine Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe.

Elham Schokraie

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## Erklärung

Ich erkläre hiermit, noch keinen Promotionsversuch unternommen zu haben.

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