6.1. Density Trace Comparison between IHHNV-Infected Prawns and the Control Prawns

The density trace is the software proofs for differential protein spots expression level between infected and non-infected samples.

1) Carbonic anhydrase 2

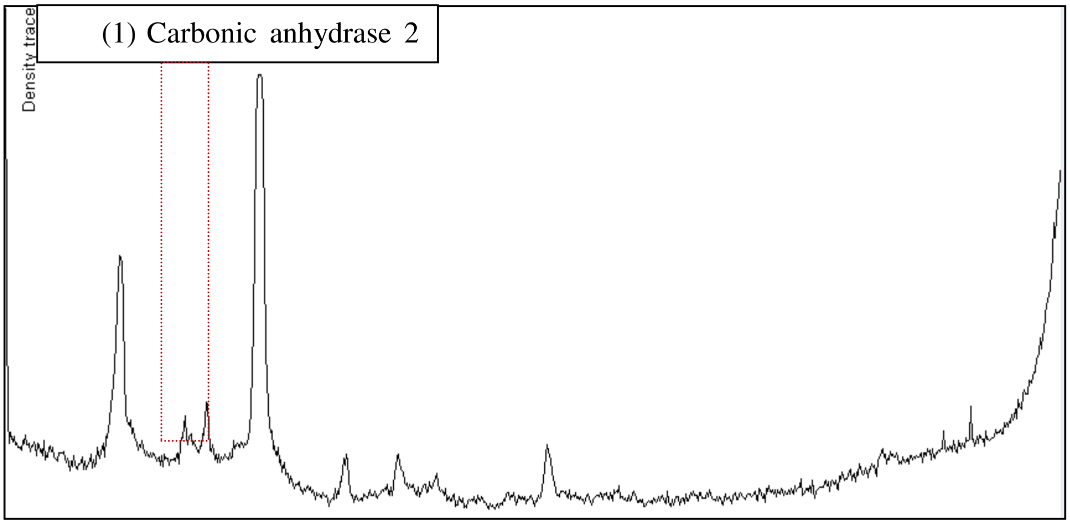

Figure 6.1, Carbonic anhydrase2 density trace in control (non-infected) sample.

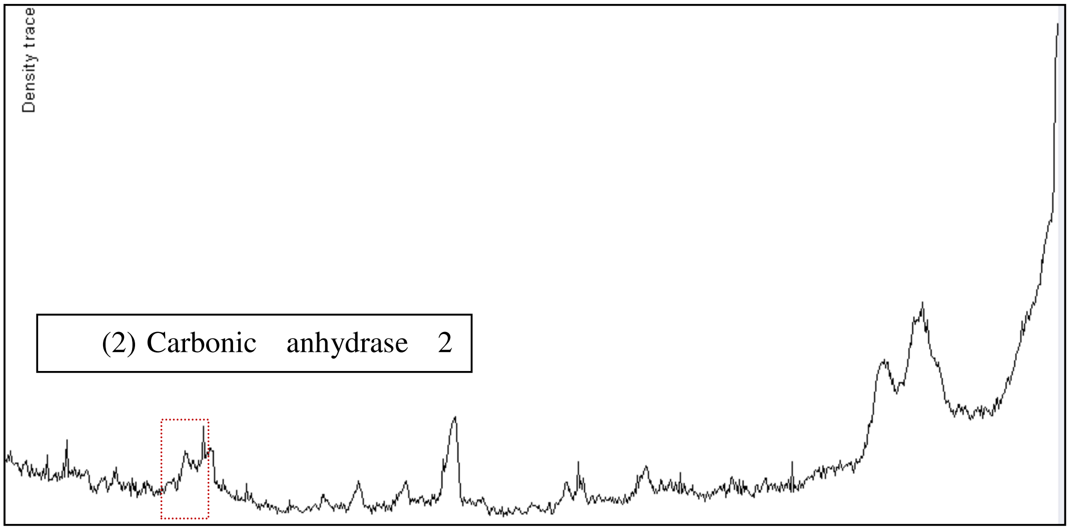

Figure 6.2, Carbonic anhydrase2 density trace in infected sample.

2) Predicted protein (Fragment)

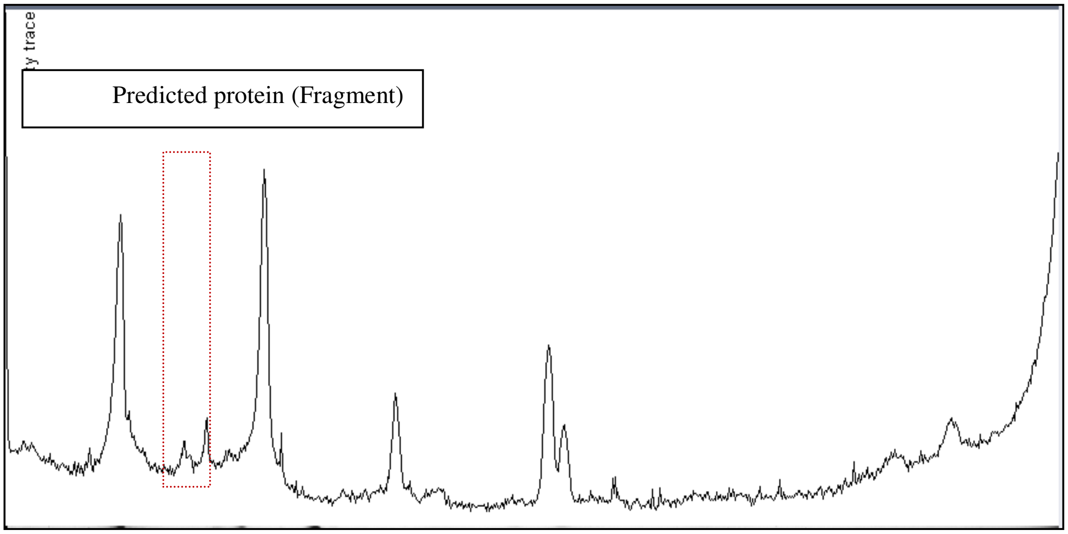

Figure 6.3, Predicted protein (Fragment) density trace in control (non-infected) sample

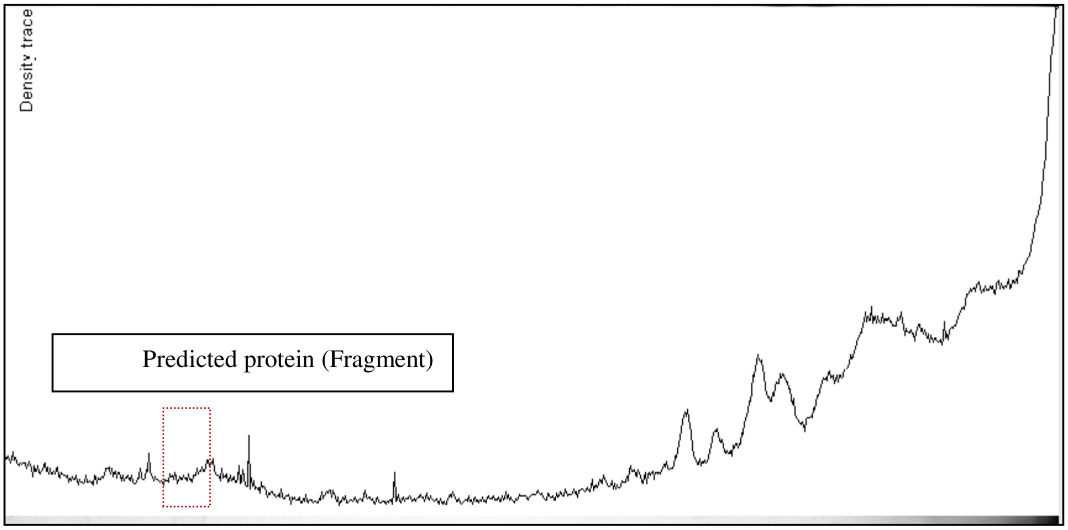

Figure 6.4, Predicted protein (Fragment) density trace in infected sample

3) Enolase

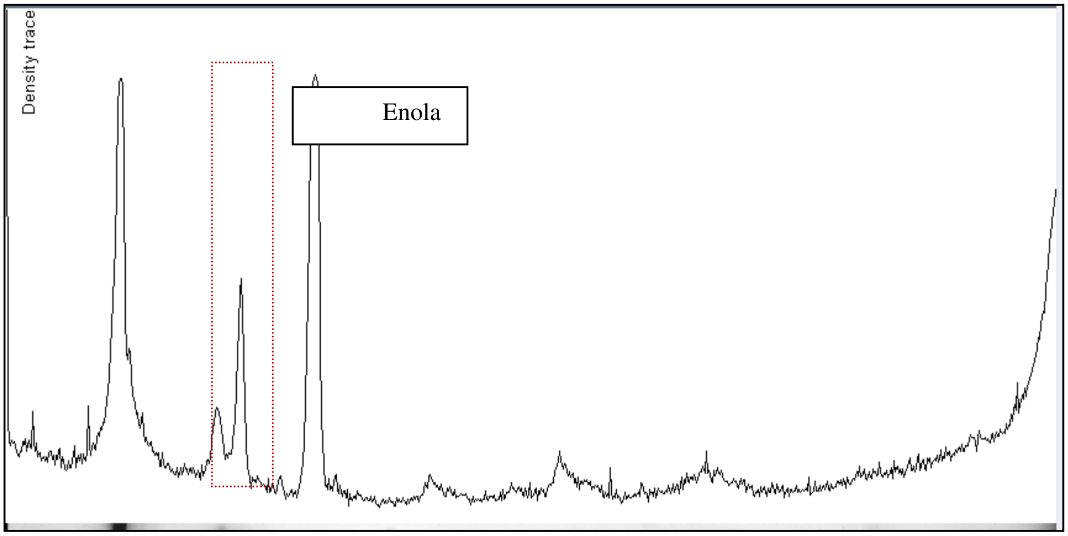

Figure 6.5, Enolase density trace in control (non-infected) sample

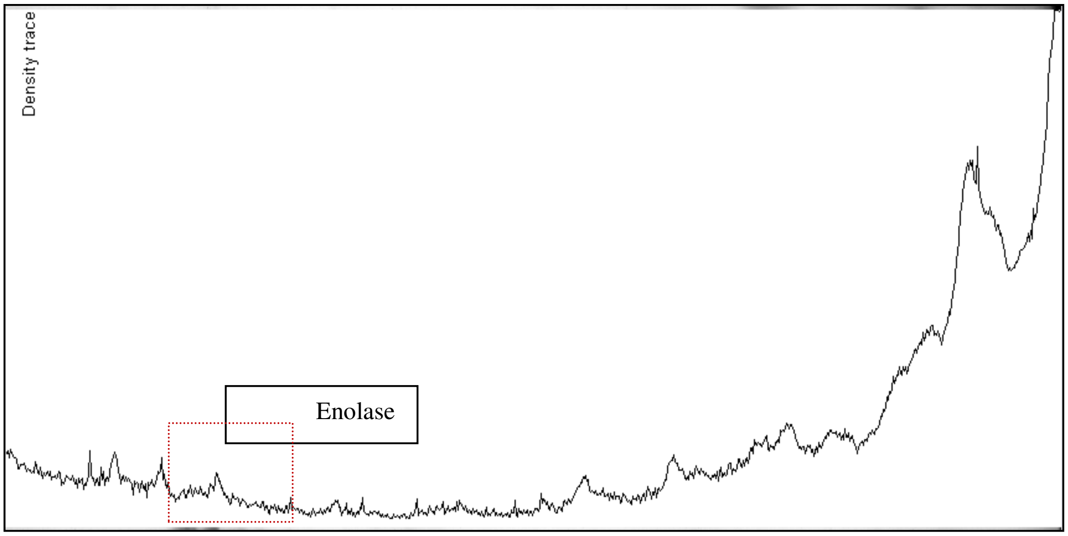

Figure 6.6, Enolase density trace in infected sample

4) Hemocyanin subunit

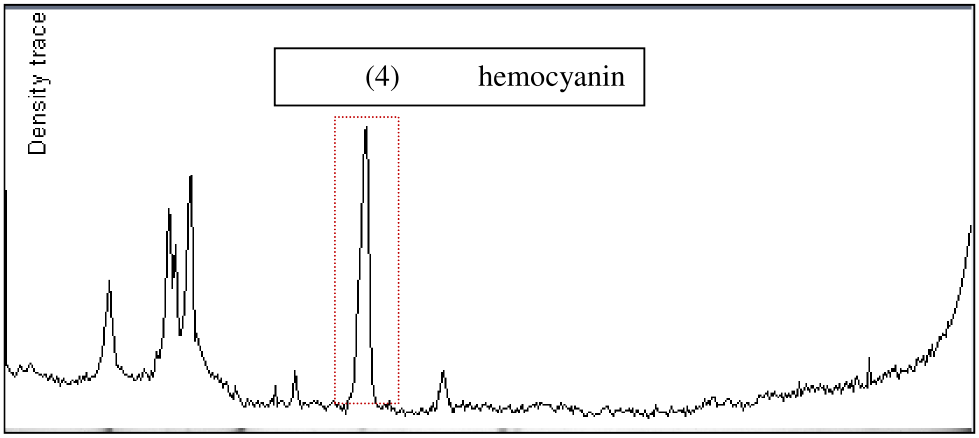

Figure 6.7, (4) hemocyanin subunit © density trace in control (non-infected) sample

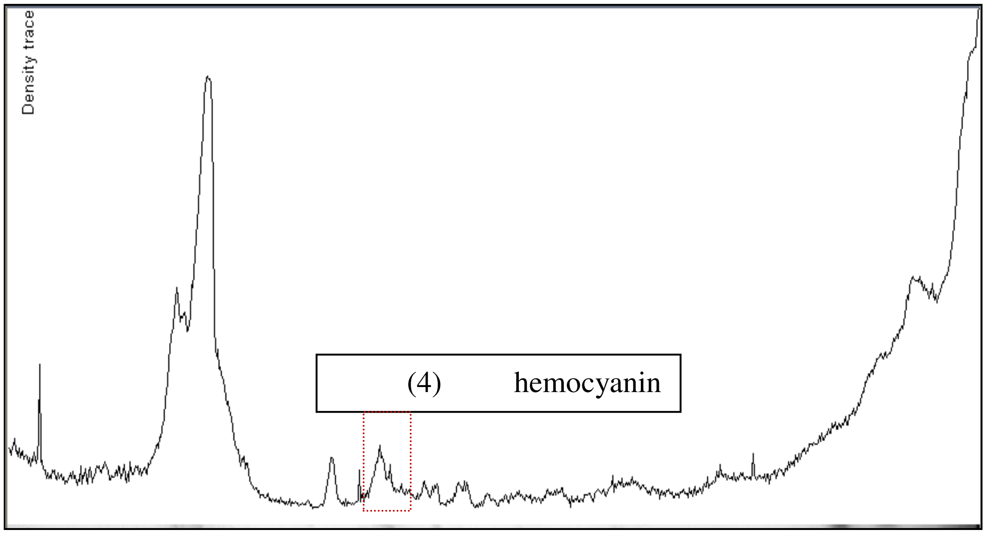

Figure 6.8, (4) hemocyanin subunit density trace in infected sample

5) Hemocyanin subunit L

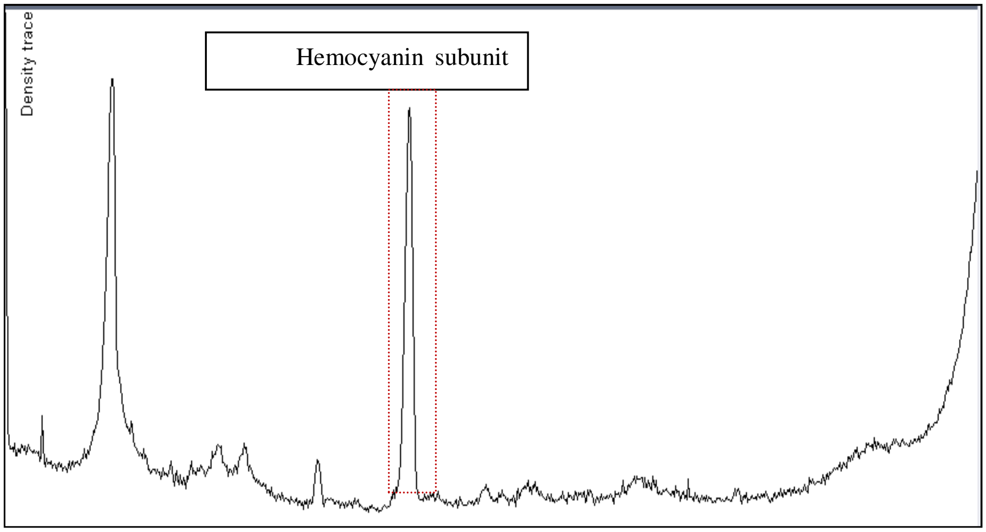

Figure 6.9, Hemocyanin subunit L © density trace in control (non-infected) sample

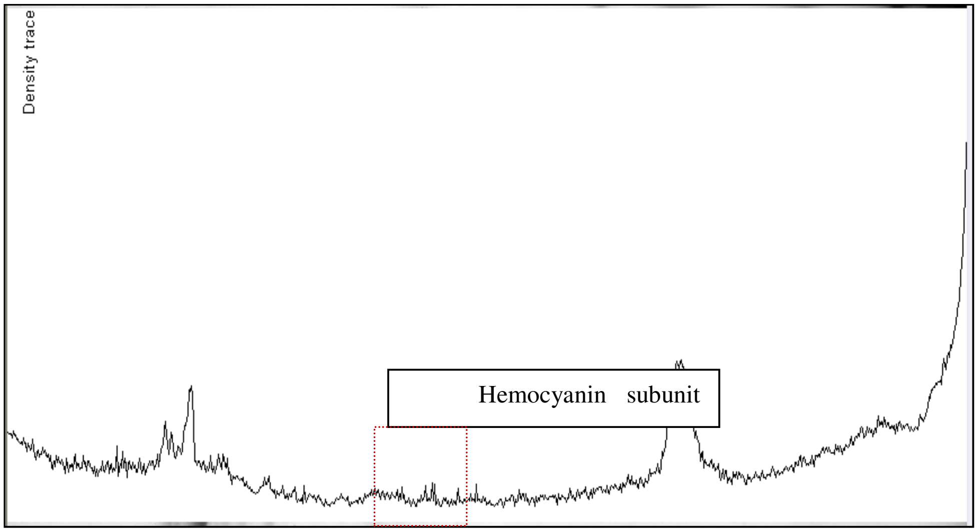

Figure 6.10, Hemocyanin subunit L density trace in infected sample

6) Hemocyanin subunit 1

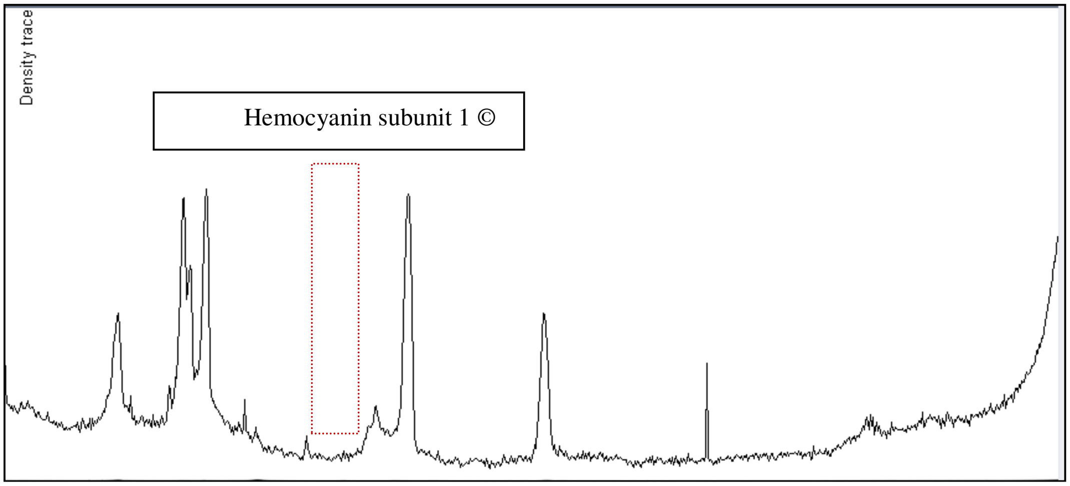

Figure 6.11, Hemocyanin subunit 1 © density trace in control (non-infected) sample

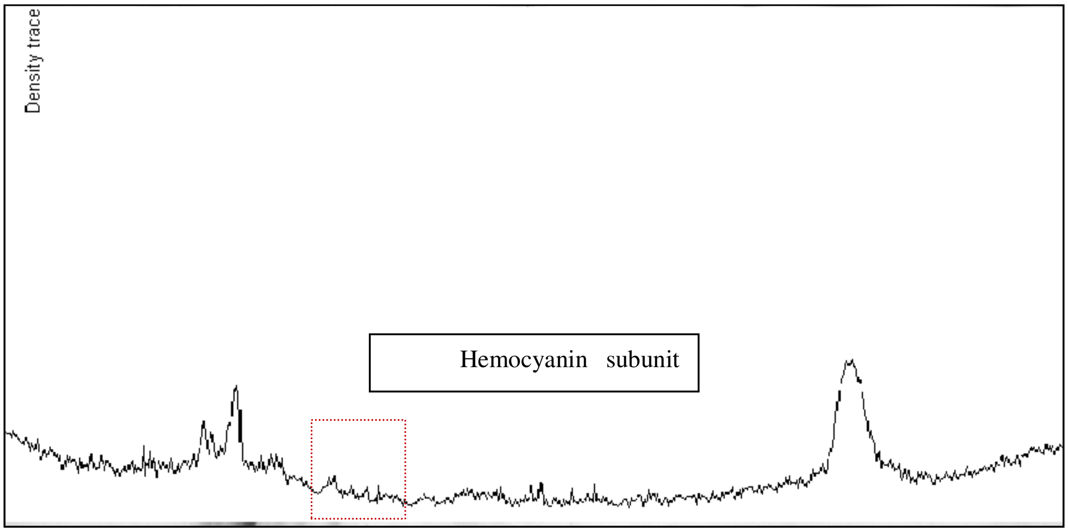

Figure 6.12, Hemocyanin subunit 1 density trace in infected sample

7) Sarcoplasmic calcium-binding protein

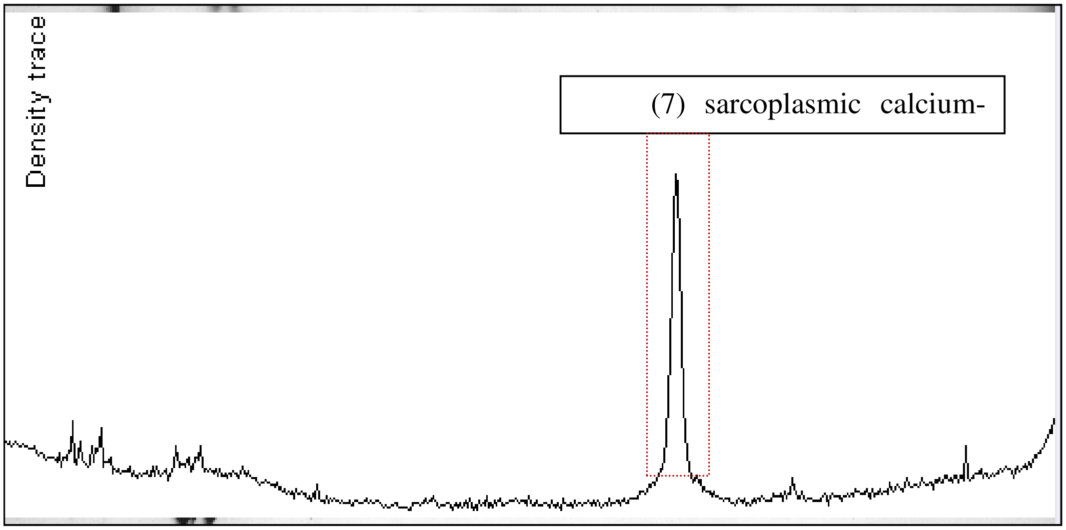

Figure 6.13, Sarcoplasmic calcium- binding © density trace in control (non-infected) sample

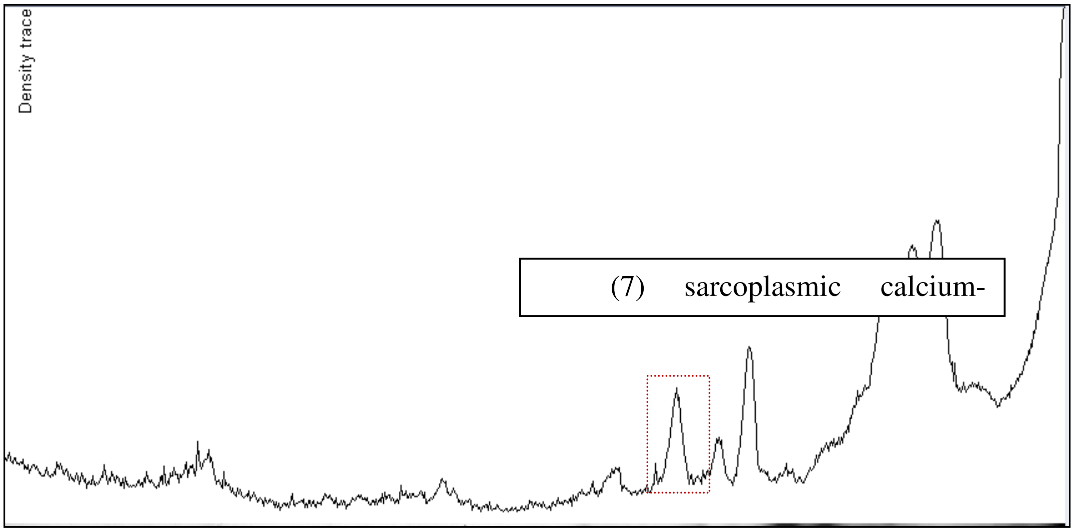

Figure 6.14, (7) sarcoplasmic calcium- binding density trace in infected sample

8-9) Arginine kinase 1

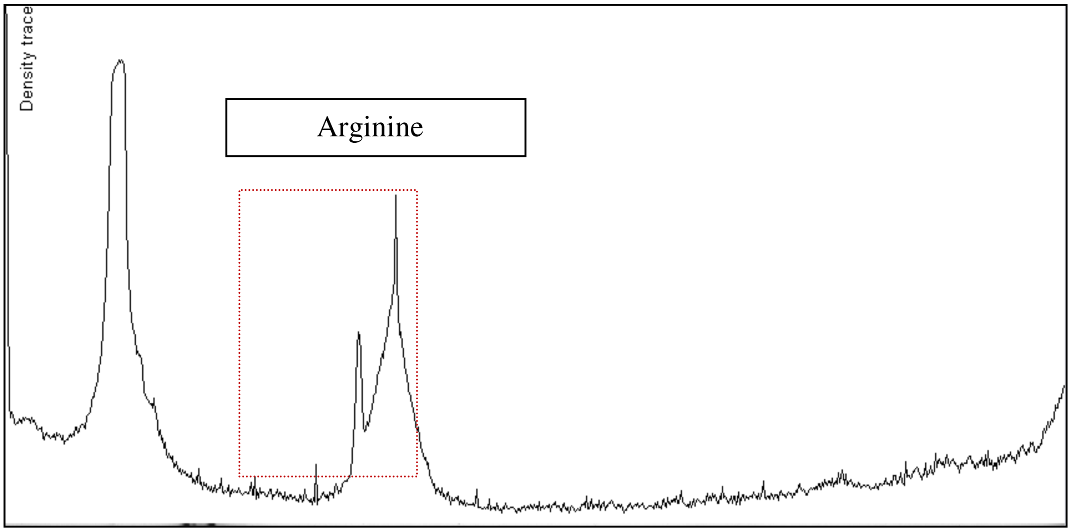

Figure 6.15, Arginine kinase1density trace in control (non-infected) sample

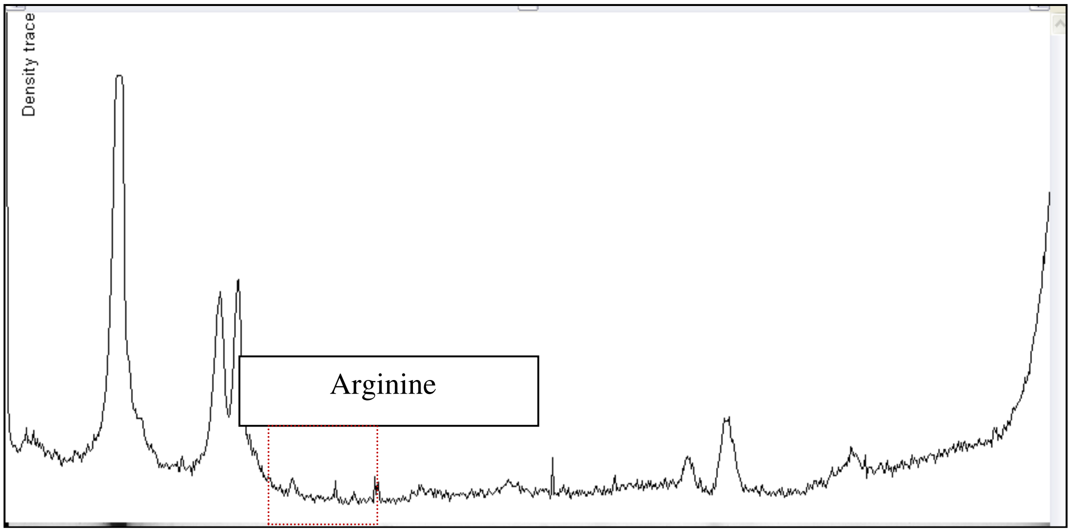

Figure 6.16, Arginine kinase 1 density trace in infected sample

10) Pro-phenoloxidase 1

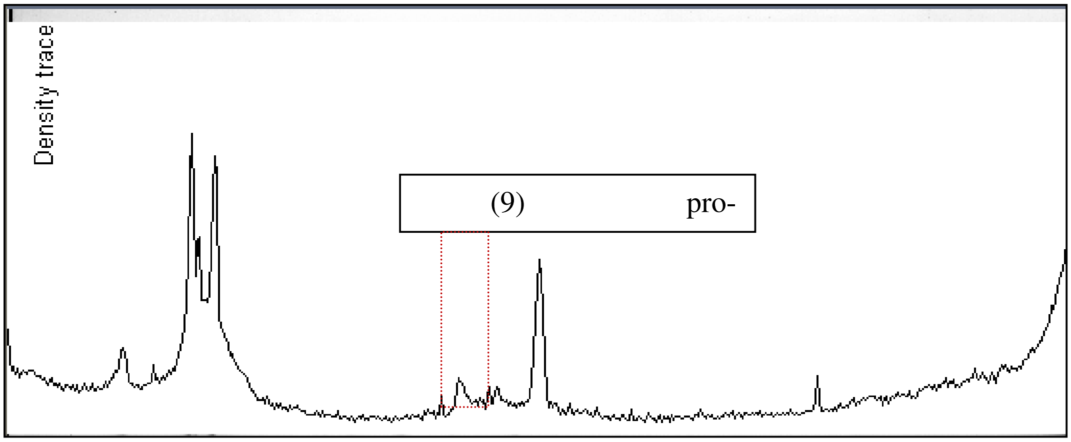

Figure 6.17, (9) pro-phenoloxidase © density trace in control (non-infected) sample

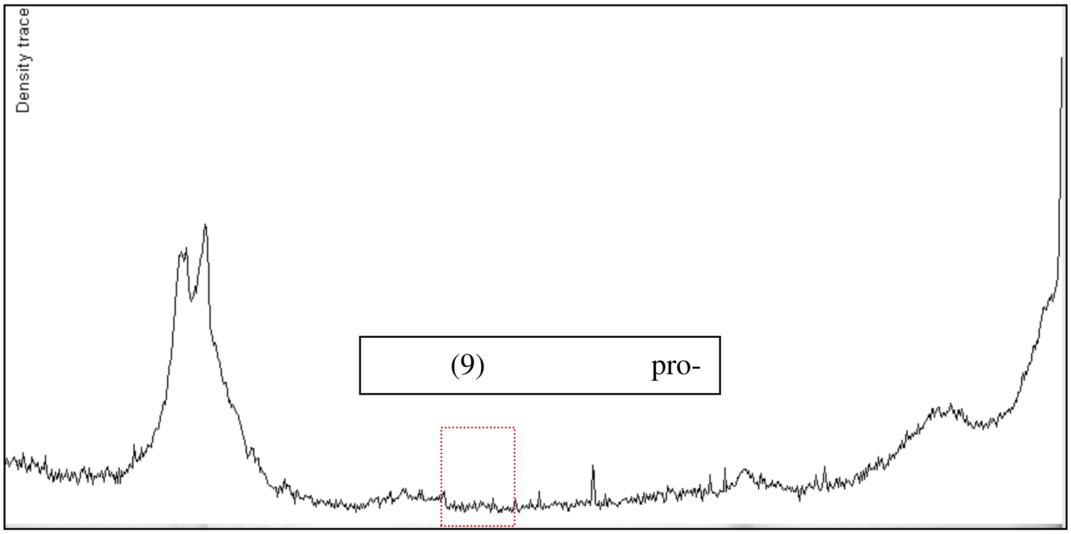

Figure 6.18, (9) pro-phenoloxidase density trace in infected sample

APPENDIX

Appendix I: Content of second Dimension: SDS PAGE solution

Table 6.1: **Acrylamide stock (30.8%)**

	Final con.	Amount
Acrylamaide	30%	87.6g
Bis		2.4g
Deonized water		Top up to 300ml
Final		*300 ml*

Table 6.2: **M TrisCL, pH8.8**

	Amount
Tris base	90.75 g
Deionized water	Top up to 250 ml
Final	*250 ml*

Table 6.3: **3. 10% (w/v) SDS**

	Amount
SDS	10g
Deionized water	100ml

Final

100ml

Table 6.4: **SDS overlay solution 0.1%**

	Amount
10% SDS solution	1ml
Deionized water	Top to 100 ml

Table 6.5: **Gel storage solution**

(0.375M)		
	Final con.	Amount
Tris-Hcl		50ml
10% SDS (w/v)		2ml
Deionized water		Top up to 200 ml

Stored at 4ºc

Table 6.6: **1X SDS Electrophoresis buffer**

(25mM)		
	Final con.	Amount
Tris		18.0g
Glycine		86.4g
SDS		6.0g
Deionized water		Top up to 6L

Stored in 4ºc

Table 6.7: **SDS equilibration buffer**

(250mM)	
	Amount
Tris-Hcl	6.70 ml
Urea	72.07g
Glycerol	68.97 ml
SDS	4.00g
Bromophenol Blue	Trace
Deionized water	200ml

Stored at -20ºc

Table 6.8: **Sealing solution**

(25mM Tris)	
	Amount
SDS Electrophoresis buffer	100 ml
Agarose M	500 mg
Bromophenol Blue	Trace

Top up to 25ml.

Table 6.9: **Rehydration buffer**

	Final conc.	Amount
Urea	8M	12.0g
CHAPS	2%	0.5g
Bromophenol blue	trace	Trace

Add 50mg DTT and 2.5µl IPG buffer before use.

Table 6.10: **Homogenous gel solution**

	Amount
Deionized water	31.8 ml
1.5M Trice-Hcl, PH 8.8	25 ml
10% SDS	1.0 ml
Acrylamaide stock 30%	41.7 ml
10% SDS 500µl	
TEMED 50µl	
Total monomer for 2 gels	100 ml

Table 6.11: **IPGphore program**

Step and voltage mod	Voltage mode	Duration(hr:min)
1 Step and hold	200	1:00
2 Step and hold	1000	1:00
3 Step and hold	8000	4: 30
4 Step and hold	8000	1
total		7: 30

Setting: 50uA/IPG strip, 20o, rehydration 0 hours

Table 6.12: **Running condition**

Phase	power		duration
1	90 V	100 W	4O min
2	250 V	120W	Until the bromophenol blue dye reaches the bottom ofthe gel.

Constant power. 2O^{o}C

SILVER STAINING FOR GEL ELECTROPHORESIS

Table 6.13: **SOLUTIONS:**

Fixing Solution	
Acetic Acid	120mL
95% Ethanol	500mL
35% Formaldehyde	500µL

* Fill up to 1L dH_2O

Sensitizing Solution	
Sodium Thiosulphate	200mg

* Fill up to 1L dH_2O

Staining Solution	
Silver Nitrate	2g
35% Formaldehyde	760µL

* Fill up to 1L dH_2O

_*_ Wrap bottle with aluminium foil and store at 20°C

_*_ Staining solution must be cold prior to use

Developing Solution	
Sodium Carbonate	60g
Sodium Thiosulphate	4mg
35% Formaldehyde	500µL

* Fill up to 1L dH_2O

Terminating Solution	
Acetic Acid	120mL

* Fill up to 1L dH_2O

4.5. Proteomics International Pty Ltd

Peptide Sequencing by MS

Report S100928SBAL

SAMPLES

Coomassie stained gel bands (n=20) were received on 22/09/2010 for Peptide sequencing by mass spectrometry analysis.

METHODS

Protein samples were trypsin digested and peptides extracted according to standard techniques (Bringans et al. Proteomics 2008). Peptides were analysed by MALDITOF- TOF mass spectrometer using a 5800 Proteomics Analyzer [AB Sciex]. Spectra were analysed to identify protein of interest using Mascot sequence matching software [Matrix Science] with Ludwig NR Database and taxonomy set to Other Metazoa.

ISO/IEC 17025. NATA accredited list of SOP's:

0_14 In-gel destain, reduction/alkylation, digestion & extraction of peptides

1_04 4800 MALDI Matrices and Sample Spotting

1_01 Operation of 4800 TOF/TOF Mass Spectrometer

1_06 Generation of Mascot MS Results

RESULTS

The results provided relate only to the samples as listed above.

When you are logged in, click “HOME” (top right side of page). Under the Mascot Utilities go to “Search Log” (if you want to see all searches just increase the number from 50 to 100 under “how many” icon). By clicking “job number” you can view your result.

See the table below to view how the samples were labeled: In the Mascot Search results, under the “Search Title” you will find the label for your sample.

For example:

QC BSA 100926-QC 132592

NOTES ON INTERPRETING THE RESULTS

Database: Ludwig NR

Ludwig NR is a comprehensive, audited database designed specifically for mass spectrometry applications. It contains non-identical protein sequence information based on all major publicly available datasets. For further information please see:

http://www.matrixscience.com/help/seq_db_setup_nr.html

Viewing the results via the weblink

Click on the weblink to see your results: “Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits” says the Mascot website.

This means protein scores are the sum of a series of peptide scores and this determines the ranking of protein hits. The probability is a measure of how unlikely it is that the hit is a random event.

Each peptide is fragmented within the mass spectrometer to produce ions that give amino acid sequence information. In each case the peptide ion data is matched to possible amino acid sequences in the database. This data frequently lends itself to more than one sequence interpretation.

1. Delta mass shows the mass difference between observed ion and matched sequence.

2. Data in **red** indicates that the protein hit ranked number one in the list of possible sequences (move the cursor over the number in the "Query" column to see the list).

3. **Black** indicates the protein hit ranked lower down the list of possible sequences. Clicking on the number in the "Query" column shows the MS/MS peptide spectra that matched the sequence.

4. When data appears in **bold** this is the first time a peptide found in your dataset has been matched to a protein.

5. Peptides not in bold are seen further down the list of hits, and show the peptide has already been matched to a protein at a higher level of significance.

6. The list of “peptide matches not assigned to protein hits” at the end of the report are sequences of low significance also contained within the sample.

In all cases the best results are achieved where two or more peptides map to the same protein. One matched peptide at high confidence is indicative. Search results are not absolute and matches near the significance threshold should be closely examined. You should be concerned if your molecular mass data does not support the Mascot hit, and a hit lower down the list but of the correct size may indicate a better match.

The results shown are generated by automatic database searching. Where no significant hit is obtained this may indicate that there is insufficient protein concentration or the protein is not in the database. Analysis against an alternative database or further *de novo* peptide sequencing may be beneficial.

The proteomics analyses were performed in facilities funded by the (WA) Lottery west State Biomedical Facility – Proteomics Node, Western Australian Institute for Medical Research, Perth, Australia. The proteomics data analyses were performed with the support of the facilities at the Australian Proteomics Computational Facility (housed at the Ludwig Institute for Cancer Research, Victoria).

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Particular attention is drawn to clause 8:

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(b) PI and the Customer hereby agree that, to the extent permitted by law, in the event of any loss, damage or claim arising out of a breach of one or more of the warranties pursuant to these Terms and Conditions, the liability of PI (if applicable) is limited to the replacement of the goods or the cost of replacement or, in the case of a service, the cost of supply or re-supply of the service.

(c) The Customer's property under PI's custody or control will be entirely at the Purchaser's risk as regards loss or damage thereto or from whatsoever cause arising.

(d) The Customer shall comply with all instructions of PI in relation to the handling, storage, sale and the use of the goods. Notwithstanding such compliance, the Customer shall keep PI indemnified against all costs, claims, demands, expenses and liabilities of whatsoever nature, including without limitation, claims for death, personal injury, damage to property and consequential loss (including loss of profit) which may be made against PI or which PI may sustain,

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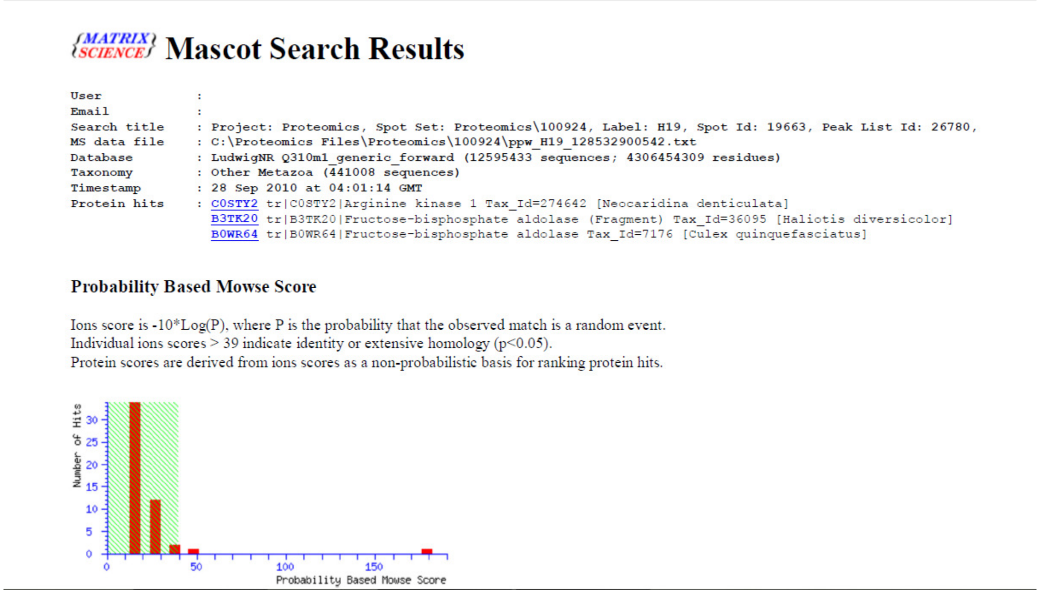
An example for mascot Result:

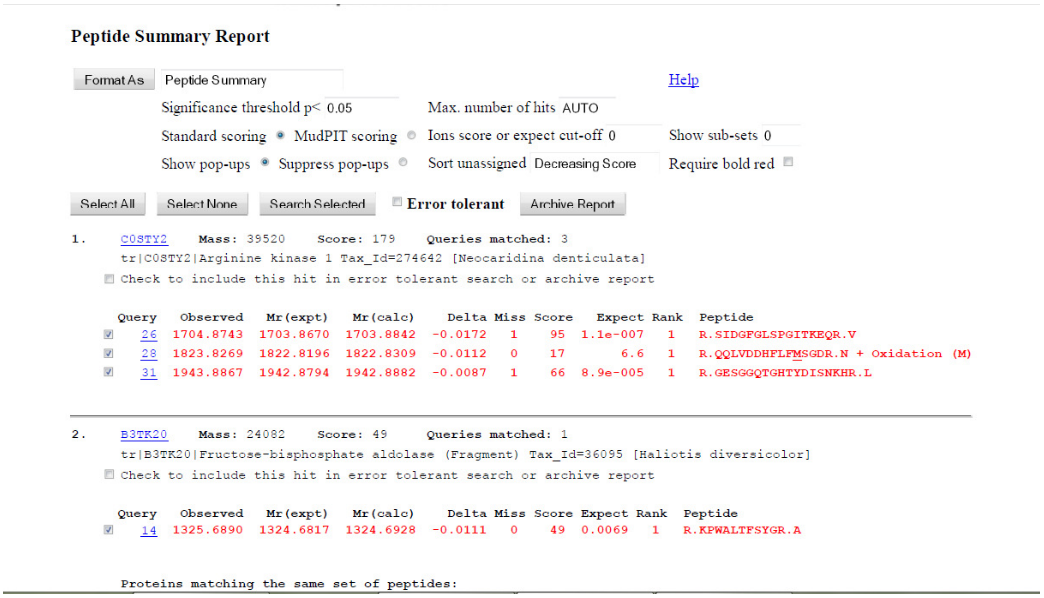


Mascot Search Results

```
User          :
Email         :
Search title  : Project: Proteomics, Spot Set: Proteomics\100924, Label: H19, Spot Id: 19663, Peak List Id: 26780,
MS data file  : C:\Proteomics Files\Proteomics\100924\ppw_H19_128532900542.txt
Database      : LudwigNR Q310ml_generic_forward (12595433 sequences; 4306454309 residues)
Taxonomy      : Other Metazoa (441008 sequences)
Timestamp     : 28 Sep 2010 at 04:01:14 GMT
Protein hits  : C0STY2 tr|C0STY2|Arginine kinase 1 Tax_Id=274642 [Neocaridina denticulata]
                B3TK20 tr|B3TK20|Fructose-bisphosphate aldolase (Fragment) Tax_Id=36095 [Haliotis diversicolor]
                B0WR64 tr|B0WR64|Fructose-bisphosphate aldolase Tax_Id=7176 [Culex quinquefasciatus]
```

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 39 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

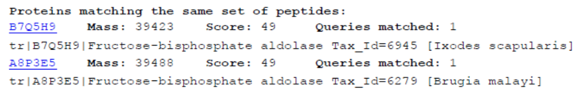


Peptide Summary Report

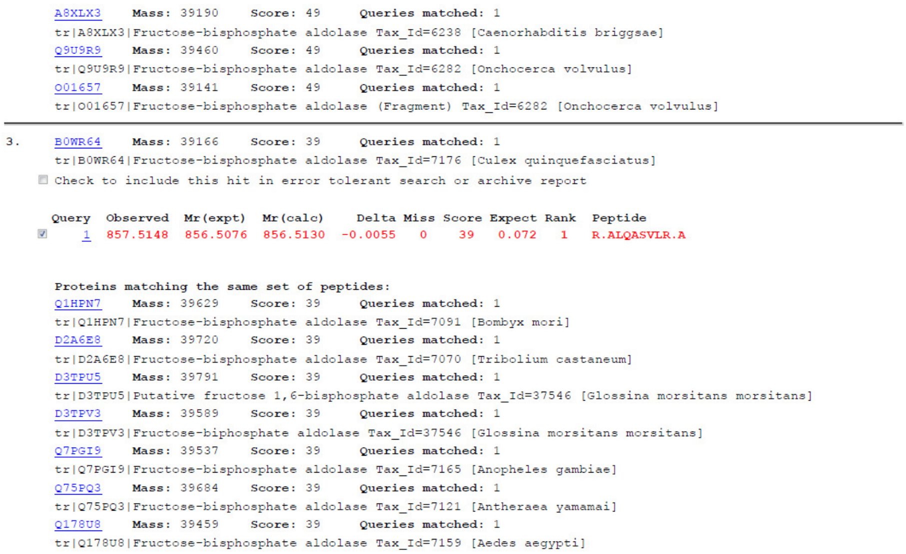
Peptide Summary
Significance threshold p< 0.05
Max. number of hits AUTO
Standard scoring ● MudPIT scoring ○
Ions score or expect cut-off 0
Show sub-sets 0
Show pop-ups ● Suppress pop-ups ○
Sort unassigned Decreasing Score
Require bold red ☐
☐ **Error tolerant**

```
1.      C0STY2     Mass: 39520     Score: 179     Queries matched: 3
        tr|C0STY2|Arginine kinase 1 Tax_Id=274642 [Neocaridina denticulata]
      ☐ Check to include this hit in error tolerant search or archive report

        Query  Observed   Mr(expt)   Mr(calc)    Delta  Miss Score  Expect  Rank  Peptide
      ☑   26  1704.8743  1703.8670  1703.8842  -0.0172   1    95  1.1e-007   1   R.SIDGFGLSPGITKEQR.V
      ☑   28  1823.8269  1822.8196  1822.8309  -0.0112   0    17       6.6   1   R.QQLVDDHFLFMSGDR.N + Oxidation (M)
      ☑   31  1943.8867  1942.8794  1942.8882  -0.0087   1    66  8.9e-005   1   R.GESGGQTGHTYDISNKHR.L
```

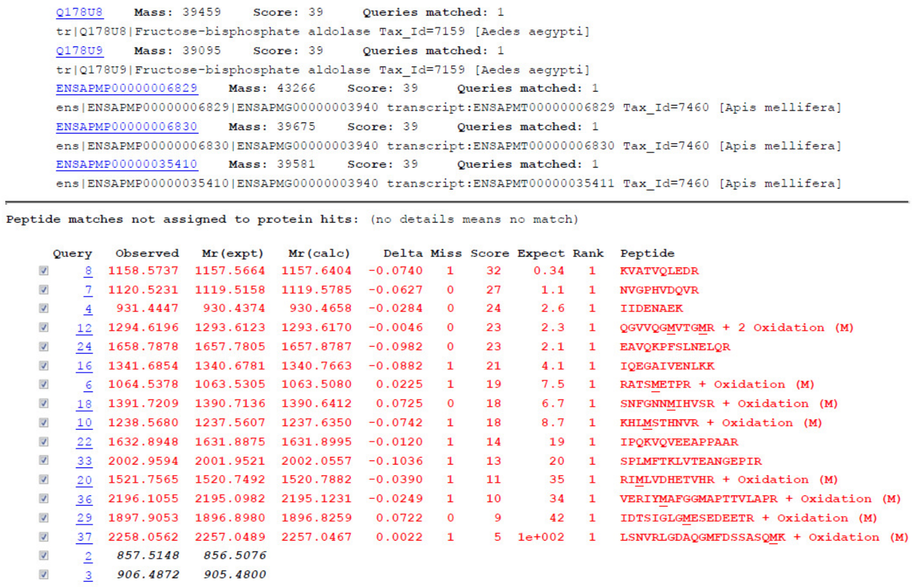
```
2.      B3TK20     Mass: 24082     Score: 49     Queries matched: 1
        tr|B3TK20|Fructose-bisphosphate aldolase (Fragment) Tax_Id=36095 [Haliotis diversicolor]
      ☐ Check to include this hit in error tolerant search or archive report

        Query  Observed   Mr(expt)   Mr(calc)    Delta  Miss Score Expect Rank  Peptide
      ☑   14  1325.6890  1324.6817  1324.6928  -0.0111   0    49  0.0069   1   R.KPWALTFSYGR.A

        Proteins matching the same set of peptides:
```



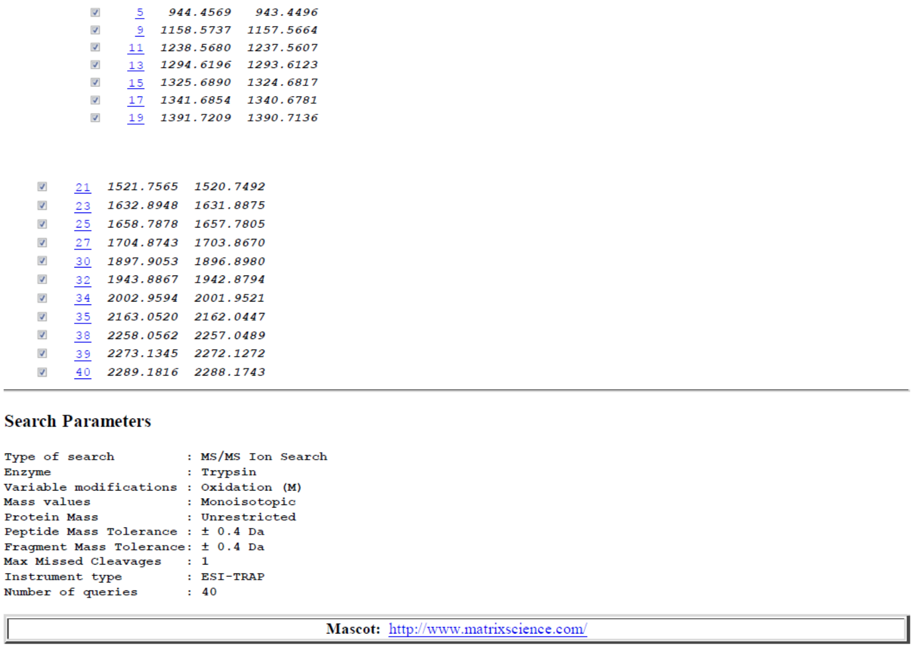
```
        Proteins matching the same set of peptides:
        B7Q5H9     Mass: 39423     Score: 49     Queries matched: 1
        tr|B7Q5H9|Fructose-bisphosphate aldolase Tax_Id=6945 [Ixodes scapularis]
        A8P3E5     Mass: 39488     Score: 49     Queries matched: 1
        tr|A8P3E5|Fructose-bisphosphate aldolase Tax_Id=6279 [Brugia malayi]
```



```
    A8XLX3     Mass: 39190    Score: 49     Queries matched: 1
    tr|A8XLX3|Fructose-bisphosphate aldolase Tax_Id=6238 [Caenorhabditis briggsae]
    Q9U9R9     Mass: 39460    Score: 49     Queries matched: 1
    tr|Q9U9R9|Fructose-bisphosphate aldolase Tax_Id=6282 [Onchocerca volvulus]
    O01657     Mass: 39141    Score: 49     Queries matched: 1
    tr|O01657|Fructose-bisphosphate aldolase (Fragment) Tax_Id=6282 [Onchocerca volvulus]
________________________________________________________________________________________

3.  B0WR64     Mass: 39166    Score: 39     Queries matched: 1
    tr|B0WR64|Fructose-bisphosphate aldolase Tax_Id=7176 [Culex quinquefasciatus]
  ☐ Check to include this hit in error tolerant search or archive report

    Query  Observed  Mr(expt)  Mr(calc)    Delta Miss Score Expect Rank  Peptide
  ☑    1   857.5148  856.5076  856.5130  -0.0055   0    39   0.072   1   R.ALQASVLR.A


    Proteins matching the same set of peptides:
    Q1HPN7     Mass: 39629    Score: 39     Queries matched: 1
    tr|Q1HPN7|Fructose-bisphosphate aldolase Tax_Id=7091 [Bombyx mori]
    D2A6E8     Mass: 39720    Score: 39     Queries matched: 1
    tr|D2A6E8|Fructose-bisphosphate aldolase Tax_Id=7070 [Tribolium castaneum]
    D3TPU5     Mass: 39791    Score: 39     Queries matched: 1
    tr|D3TPU5|Putative fructose 1,6-bisphosphate aldolase Tax_Id=37546 [Glossina morsitans morsitans]
    D3TPV3     Mass: 39589    Score: 39     Queries matched: 1
    tr|D3TPV3|Fructose-biphosphate aldolase Tax_Id=37546 [Glossina morsitans morsitans]
    Q7PGI9     Mass: 39537    Score: 39     Queries matched: 1
    tr|Q7PGI9|Fructose-bisphosphate aldolase Tax_Id=7165 [Anopheles gambiae]
    Q75PQ3     Mass: 39684    Score: 39     Queries matched: 1
    tr|Q75PQ3|Fructose-bisphosphate aldolase Tax_Id=7121 [Antheraea yamamai]
    Q178U8     Mass: 39459    Score: 39     Queries matched: 1
    tr|Q178U8|Fructose-bisphosphate aldolase Tax_Id=7159 [Aedes aegypti]
```



```
    Q178U8     Mass: 39459    Score: 39     Queries matched: 1
    tr|Q178U8|Fructose-bisphosphate aldolase Tax_Id=7159 [Aedes aegypti]
    Q178U9     Mass: 39095    Score: 39     Queries matched: 1
    tr|Q178U9|Fructose-bisphosphate aldolase Tax_Id=7159 [Aedes aegypti]
    ENSAPMP00000006829     Mass: 43266    Score: 39     Queries matched: 1
    ens|ENSAPMP00000006829|ENSAPMG00000003940 transcript:ENSAPMT00000006829 Tax_Id=7460 [Apis mellifera]
    ENSAPMP00000006830     Mass: 39675    Score: 39     Queries matched: 1
    ens|ENSAPMP00000006830|ENSAPMG00000003940 transcript:ENSAPMT00000006830 Tax_Id=7460 [Apis mellifera]
    ENSAPMP00000035410     Mass: 39581    Score: 39     Queries matched: 1
    ens|ENSAPMP00000035410|ENSAPMG00000003940 transcript:ENSAPMT00000035411 Tax_Id=7460 [Apis mellifera]
________________________________________________________________________________________
Peptide matches not assigned to protein hits: (no details means no match)

    Query  Observed   Mr(expt)   Mr(calc)    Delta Miss Score Expect Rank  Peptide
  ☑    8  1158.5737  1157.5664  1157.6404  -0.0740   1    32    0.34   1   KVATVQLEDR
  ☑    7  1120.5231  1119.5158  1119.5785  -0.0627   0    27     1.1   1   NVGPHVDQVR
  ☑    4   931.4447   930.4374   930.4658  -0.0284   0    24     2.6   1   IIDENAEK
  ☑   12  1294.6196  1293.6123  1293.6170  -0.0046   0    23     2.3   1   QGVVQGMVTGMR + 2 Oxidation (M)
  ☑   24  1658.7878  1657.7805  1657.8787  -0.0982   0    23     2.1   1   EAVQKPFSLNELQR
  ☑   16  1341.6854  1340.6781  1340.7663  -0.0882   1    21     4.1   1   IQEGAIVENLKK
  ☑    6  1064.5378  1063.5305  1063.5080   0.0225   1    19     7.5   1   RATSMETPR + Oxidation (M)
  ☑   18  1391.7209  1390.7136  1390.6412   0.0725   0    18     6.7   1   SNFGNNMIHVSR + Oxidation (M)
  ☑   10  1238.5680  1237.5607  1237.6350  -0.0742   1    18     8.7   1   KHLMSTHNVR + Oxidation (M)
  ☑   22  1632.8948  1631.8875  1631.8995  -0.0120   1    14      19   1   IPQKVQVEEAPPAAR
  ☑   33  2002.9594  2001.9521  2002.0557  -0.1036   1    13      20   1   SPLMFTKLVTEANGEPIR
  ☑   20  1521.7565  1520.7492  1520.7882  -0.0390   1    11      35   1   RIMLVDHETVHR + Oxidation (M)
  ☑   36  2196.1055  2195.0982  2195.1231  -0.0249   1    10      34   1   VERIYMAFGGMAPTTVLAPR + Oxidation (M)
  ☑   29  1897.9053  1896.8980  1896.8259   0.0722   0     9      42   1   IDTSIGLGMESEDEETR + Oxidation (M)
  ☑   37  2258.0562  2257.0489  2257.0467   0.0022   1     5  1e+002   1   LSNVRLGDAQGMFDSSASQMK + Oxidation (M)
  ☑    2   857.5148   856.5076
  ☑    3   906.4872   905.4800
```



```
☑   5    944.4569    943.4496
☑   9   1158.5737   1157.5664
☑  11   1238.5680   1237.5607
☑  13   1294.6196   1293.6123
☑  15   1325.6890   1324.6817
☑  17   1341.6854   1340.6781
☑  19   1391.7209   1390.7136


☑  21   1521.7565   1520.7492
☑  23   1632.8948   1631.8875
☑  25   1658.7878   1657.7805
☑  27   1704.8743   1703.8670
☑  30   1897.9053   1896.8980
☑  32   1943.8867   1942.8794
☑  34   2002.9594   2001.9521
☑  35   2163.0520   2162.0447
☑  38   2258.0562   2257.0489
☑  39   2273.1345   2272.1272
☑  40   2289.1816   2288.1743
```

Search Parameters

```
Type of search         : MS/MS Ion Search
Enzyme                 : Trypsin
Variable modifications : Oxidation (M)
Mass values            : Monoisotopic
Protein Mass           : Unrestricted
Peptide Mass Tolerance : ± 0.4 Da
Fragment Mass Tolerance: ± 0.4 Da
Max Missed Cleavages   : 1
Instrument type        : ESI-TRAP
Number of queries      : 40
```

Mascot: http://www.matrixscience.com/

Figure 6.19, An example of MS/MS Result

OUTPUT FROM CURRENT RESEARCH:

Oral presentations:

Differential expression proteins in Infected and Non Infected Infectious Hypodermal and hematopoietic necrosis virus (IHHNV) in Malaysian wild *Macrobrachium rosenbergii.*

Alinejad T1,2, Vejayan, J. 3, Othman,R.Y1,2 and Bhassu, S. 1,2

1. Genetics and Molecular Biology Div., Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

2. Centre for Research in Biotechnology for Agriculture, CEBAR, University of Malaya Kuala Lumpur, Malaysia

3School of Medicine and Health sciences, Monash University Sunway Campus, Jalan Lagoon Selatan, 46150 Subang Jaya, Selangor Darul Ehsan, Malaysia.

35th ANNUAL CONFERENCE OF THE MALAYSIAN SOCIETY FOR BIOCHEMISTRY & MOLECULAR BIOLOGY 27th -28th July 2010, Equatorial Hotel, Bangi Malaysia.

Poster presentation:

Preliminary Proteomic Characterizations of *Macrobrachium rosenbergii* hemolymph

Alinejad, T1,2. Vejayan, J. 3, Othman, R.Y1,2 and Bhassu, S. 1,2

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3.School of Medicine and Health sciences, Monash University Sunway Campus, Jalan Lagoon Selatan, 46150 Subang Jaya, Selangor Darul Ehsan, Malaysia.

Human Proteome Organization(HUPO) , 19-23 September 2010 Sydney convention and exhibition center.

Paper:

Differential expression of proteins from wild giant freshwater prawn *Macrobrachium rosenbergii* infected with infectious hypodermal and hematopoitic necrosis (IHHNV)