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*Comparative proteomics of excretory-secretory proteins released by the liver fluke *Fasciola hepatica* in sheep host bile and during in vitro culture ex host*

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Table 1. Unique peptides hit during PMF to assign a specific cathepsin L entry to a single spot

Spot Number	M _r ⁺ Submitted	M _r ⁺ Matched	Sequence	Accession Number	Description
1	2449.07	2449.07	DQGQCGSCWAFSTTGAVEGQFR	CAA80446	Cathepsin L-like Proteinase
2	2448.06	2448.05	NQGQCGSCWAFSTTGAVEGQFR	AAC47721	Secreted Cathepsin L2
5	1241.54	1241.53	NSWGSYWGER	AAM11647	Cathepsin L

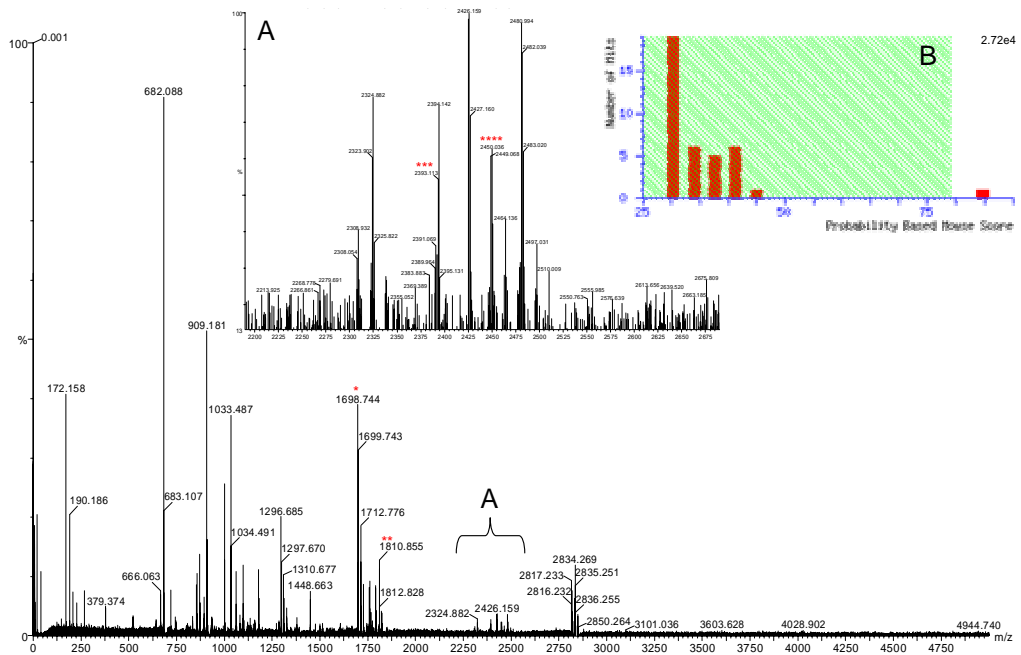


Figure 1. A representative MALDI-ToF PMF from spot 1; top hit *F. hepatica* Cathepsin L-like proteinase, CAA80446. Red stars correspond to the following peptides *ASASFSEQLVDCTR, **VTGYTVHSGDEIELK, ***LTHAVLAVGYSQDGTDYWIWK and ****DQGQCGSCWAFSTTGAVEGQFR. Shown inset (A) is an enlarged section of the same spectrum showing the location of the unique peptide (****) used to identify this specific cathepsin L (See also table 1). Shown inset (B) is the Mascot indication of significance for the hit at P = 1%.

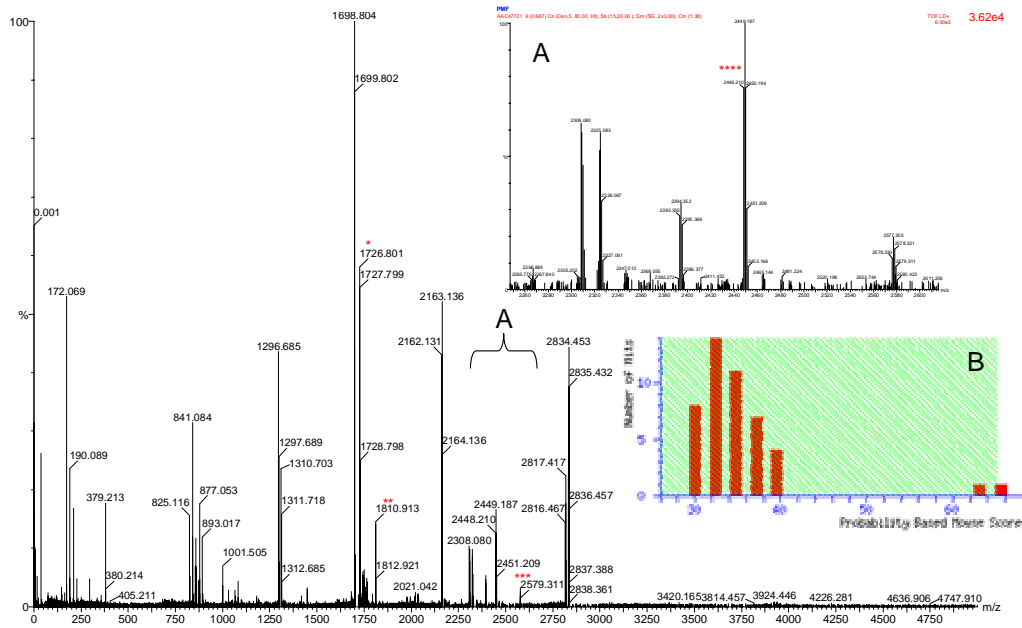


Figure 2. A representative MALDI-ToF PMF from spot 2; top hit *F. hepatica* Secreted Cathepsin L2, AAC47721. Red stars correspond to the following peptides *NSWGTWWGEDGYIR, **VTGYTVHSGDEIELK, ***NQGQCGSCWAFSTTGAVEGQFRK (1 Missed cleavage) and ****NQGQCGSCWAFSTTGAVEGQFR. Shown inset (A) is an enlarged section of the same spectrum showing the location of the unique peptide (****) used to identify this specific cathepsin L (See also table 1). Shown inset (B) is the MASCOT indication of significance for the hit at P = 5%.

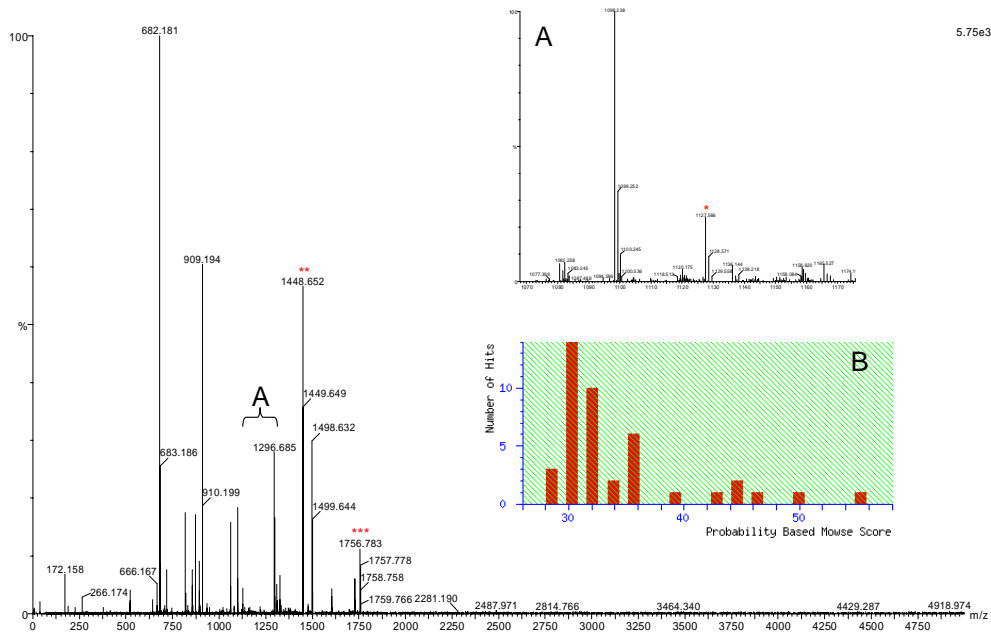


Figure 3. A representative MALDI-ToF PMF from spot 3; top hit *F. hepatica* Cathepsin L, AAF76330. Red stars correspond to the following peptides *AVPDRIDWR, **TSISFSEQLVDCSR and ***FGLEESSYPYR. Shown inset (A) is an enlarged section of the same spectrum detailing a peptide (*) that is identical to AAF76330 and AAA29137 only. The peptide at m/z 1448.652 (**) has identical matches to AAF76330, CAA80447 and AAB41670 only. Therefore the combination of these two peptides in the PMF profile helps to significantly identify this cathepsin L at this location. Shown inset (B) is the MASCOT indication for the top hit (score 55) at a significance level of P = 5%. Although not significant it was confirmed as a cathepsin L.

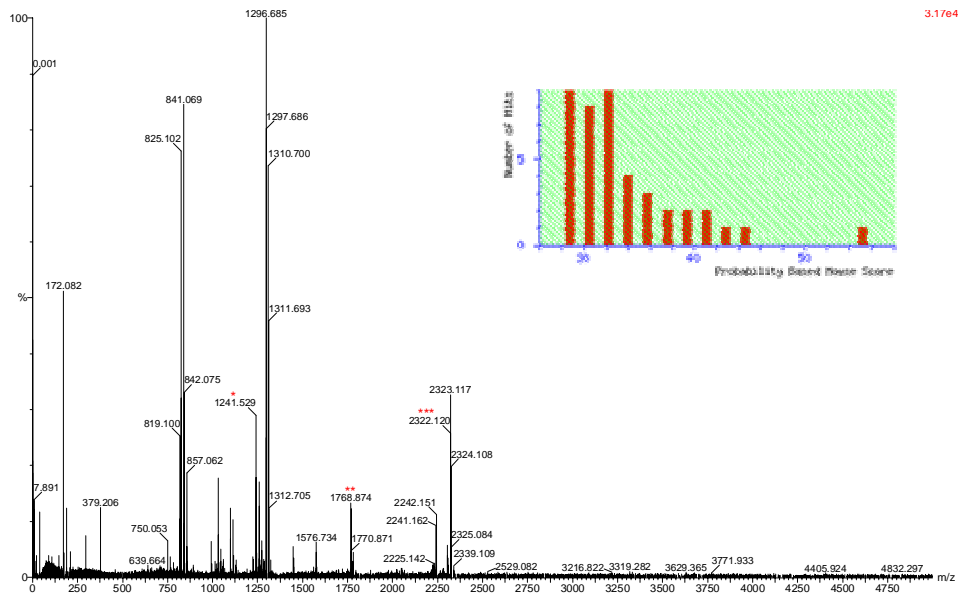


Figure 4. A representative MALDI-ToF PMF from spot 4; top hit *F. hepatica* Cathepsin L-like proteinase, AAK38169. Red stars correspond to the following peptides *NDDLWHQWK (N-terminal of Sequence in Propeptide 1241.53, *m/z* Matched 1241.56), **VTGYTVHSGSEVELK and ***QFGLETESSYPYTAVEGQCR. Shown inset is the MASCOT indication for the top hit (score 55) at a significance level of P = 5%. Although not significant it was confirmed as a cathepsin L.

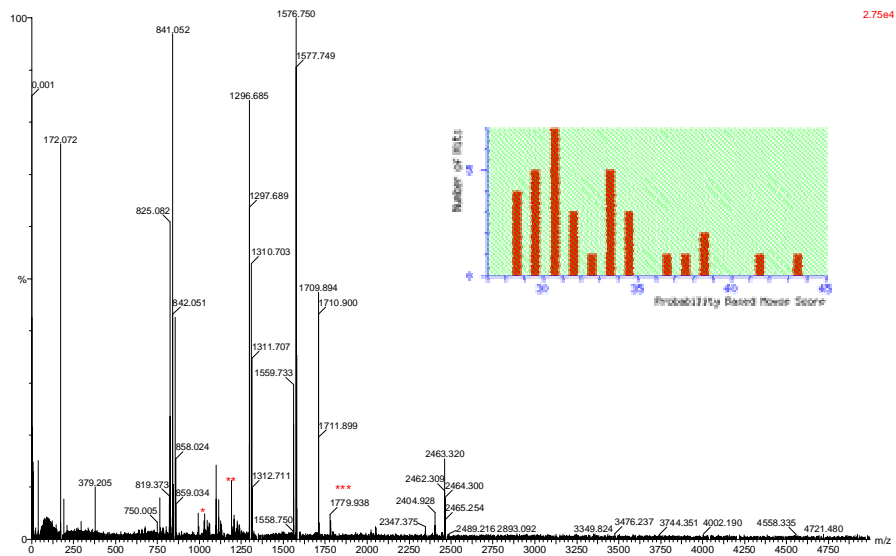


Figure 5. A representative MALDI-ToF PMF from spot 5; top hit *F. gigantica* Cathepsin L, AAF44678. Red stars correspond to the following peptides *VPDKIDWR N-terminal sequence (*m/z* 1028.552), **NSWGSSWGER (Acetyl N-term) and ***GNMCGIASLASLPMVAR. Shown inset is the MASCOT indication for the top hit (score 44) at a significance level of P = 5%. Although not significant it was confirmed as a cathepsin L.

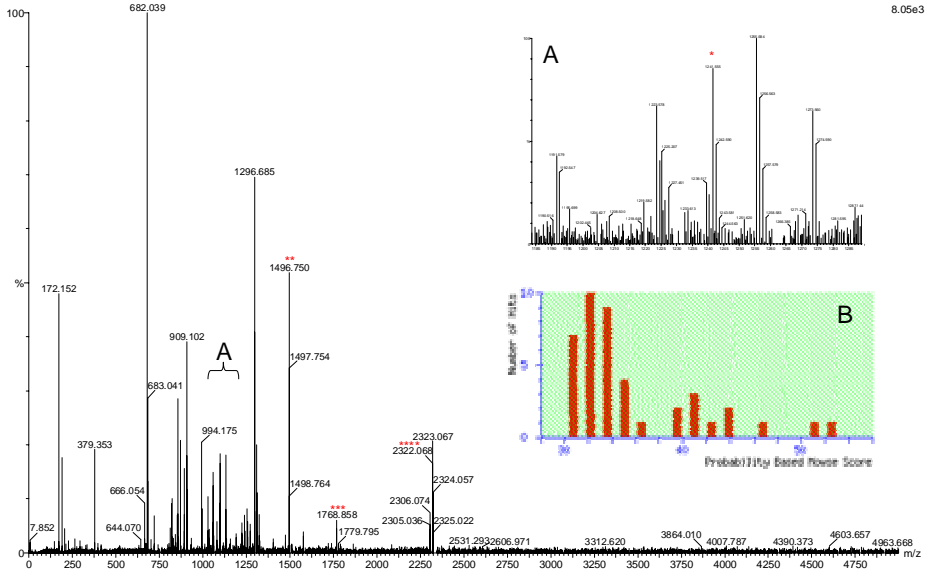


Figure 6. A representative MALDI-ToF PMF from spot 6; top hit *F. hepatica* Cathepsin L, AAM11647. Red stars correspond to the following peptides *NSWGSYWGER (Unique to AAM11647, see table 1), **SGIYQSQTCSPLR, ***VTGYTVHSGSEVELK and ****QFLETSSYPYTAVEGQCR. Shown inset (A) is an enlarged section of the same spectrum highlighting the unique peptide (*) distinctly identifying this cathepsin L. Shown inset (B) is the MASCOT indication for the top hit (score 53) at a significance level of P = 5%. Although not significant it was confirmed as a cathepsin L.

AAA29137	MRLVILTLITVGVFASNDLWQWKRIVNKEYKADLDRRRNIWEQNVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGIP
AAF76330	MRLVILTLITVGVFASNDLWQWKRIVNKEYKADLDRRRNIWEQNVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGIP
AAF49831	MWFFVLAVLITVGVLSNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGVP
BAA23743	MRLFLAVLITVGVLSNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGIP
AAA29136	MRLFLAVLITVGVLSNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGVP
AAR99518	MRLFLAVLITVGVLSNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGVP
AAB41670	MRLFLAVLITVGVLSNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGVP
AAC47721	MRCFVLAVLITVGVASNDLWQWKRIVNKEYKADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDLTFEFPKAKYLITMFRASEILSRGIP
Q24940	MRLFLAVLITVGVLSNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGVP
AAT76664	MRLFLAVLITVGVLSNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDLTFEFPKAKYLITMFRASEILSHGIP
CAA80446	MRFFVLAVLITVGVFASNDLWQWKRIVNKEYKADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDLTFEFPKAKYLITMFRASEILSRGIP
AAM11647	-----SNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGVP
CAC12806	-----SNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGIP
CAC12805	-----SNTVSNHEWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDLTFEFPKAKYLITMFRASEILSHGIP
P80528	-----SNTVSNHEWKRMYNKEYNGA
AAK38169	-----SNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDMTFEFPKAKYLITMFRASEILSHGIP
CAC12807	-----MHEWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDLTFEFPKAKYLITMFRASEILSHGIP
AAR99519	-----SNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDLTFEFPKAKYLITMFRASEILSHGIP
AAB29986	-----SNDDLWQWKRMYNKEYNGADLQHRRNIVEENVKHIQEHNLRHDLGLVITYKGLNQPTDLTFEFPKAKYLITMFRASEILSHGIP
Q09093	-----
P80532	-----
AAB35021	-----
P80342	-----
CAA80450	-----
CAA80448	-----
CAA80447	-----
CAA80445	-----
CAA80444	-----

Figure 7. Sequence alignment of all *F. hepatica* cathepsin L sequences within Genbank (<http://www.ncbi.nlm.nih.gov/>); aligned using Clustal W through BioEdit Version 7.0.5.3 (10/28/05). Boxed in blue is the peptide sequence from AAK38169 (Mr 1241) matched during MASCOT analysis which lies within the propeptide region of the sequence. Boxed in red are peptide sequences hit during PMF analysis corresponding to unique peptides for sequences they come from. The dotted line indicates the N-terminus of the mature enzymes after the signal/pre and pro peptides have been cleaved.

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      |
AAA29137 YKANKRAVDPDRIDWRESGYVTEVKDQGCSSCWAFFSTTGAMEQYMKNEKTSISFSEQLVDCSFPNGYGCNGGLMENAYEYLKRPGLTETESSYPYRAV
AAF76330 YKANKRAVDPDRIDWRESGYVTEVKDQGCSSCWAFFSTTGAMEQYMKNEKTSISFSEQLVDCSFPNGYGCNGGLMENAYEYLKRPGLTETESSYPYRAV
AAP49831 YETNNRAVDPDKIDWRESGYVTEVKDQGCSSCWAFFSTTGMEQYMKNERISISFSEQLVDCSFPWNGGCGGLMENAYCYLKPGLTETESSYPYRAV
BAA23743 YEANNRAVDPDKIDWRESGYVTEVKDQGCSSCWAFFSTTGMEQYMKNERISISFSEQLVDCSFPWNGYGCNGGLMENAYEYLKRPGLTETESSYPYRAV
AAA29136 YEANNRAVDPDKIDWRESGYVTEVKDQGCSSCWAFFSTTGMEQYMKNERISISFSEQLVDCSFPWNGGCGGLMENAYCYLKPGLTETESSYPYRAV
AAR99518 YETNNRAVDPDKIDWRESGYVTEVKDQGCSSCWAFFSTTGMEQYMKNERISISFSEQLVDCSFPWNGGCGGLMENAYCYLKPGLTETESSYPYRAV
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AAC47721 FKANKLAVPESLDWRCYVYVTEVVDGCGCCGWAFFSTTGAVEGQFRINERASASFSSEQLVDCRDLRANQCQGGYMENAYEYLKHNGLTETESSYPYQAV
Q24940 YEANNRAVDPDKIDWRESGYVTEVKDQGCSSCWAFFSTTGMEQYMKNERISISFSEQLVDCSFPWNGGCGGLMENAYCYLKPGLTETESSYPYRAV
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CAA80446 YKANKRAVPELDWRCYVYVTEVVDGCGCCGWAFFSTTGAVEGQFRINERASASFSSEQLVDCRDLRANQCQGGYMENAYEYLKHNGLTETESSYPYQAV
AAM11647 YEANNRAVDPDKIDWRESGYVTEVKDQGCSSCWAFFSTTGTEQYMKNERISISFSEQLVDCSFPWNGGCGGLMENAYCYLKPGLTETESSYPYRAV
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P80528 -----|-----
AAK38169 YEANNRAVDPDKIDWRESGYVTEVKDQGCSSCWAFFSTTGMEQYMKNERISISFSEQLVDCSFPWNGGCGGLMENAYCYLKPGLTETESSYPYRAV
CAC12807 YEAEQNDVBSIDWRCYGVVTEVKDQGCSSCWAFFSVAIGEQYVKKFQNCITLSESEQLVDCRTRFPNGHCCGGWMMENAYKYLKNSGLTETASSYPYQAV
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AAB29986 -----|AVPDKIDPRESGYVTEVKDQ-----
Q09093 -----|AVPDKIDPRESGYVTEVKDQ-----
P80532 -----|VPEASIDWREYGVVTEVKD-----
AAB35021 -----|VPEASIDWREYGVVTEVKD-----
P80342 -----|AVPDKIDPRESGYV-----
CAA80450 -----|-----CGCCGWAFFSTTGMEQYMKKCRISISFSEQLVDCSRFPWNGGCGGLMENAYCYLKPGLTETESSYPYRAV
CAA80448 -----|-----CGCCGWAFFSTTGVEGQYSRKYGSTLGFSEQLVDCRFRHNEGCGGLMNTSSYVILMNSGLESCDYVPEAM
CAA80447 -----|-----CGCCGWAFFSTTGATEQYMKNERISISFSEQLVDCSRFPNGYGCNGGLMENAYEYLKRPGLTETESSYPYRAV
CAA80445 -----|-----CGCCGWAFFSTTGAVEGQFRKNERASASFSSEQLVDCRDLRANQCQGGYMENAYEYLKHNGLTETESSYPYQAV
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Figure 7. Continued

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AAA29137 EGQCRYNQLGVAKVTGYVTVHSGDEVELQNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
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AAP49831 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
BAA23743 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
AAA29136 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
AAR99518 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
AAB41670 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
AAC47721 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
Q24940 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
AAT76664 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
CAA80446 EGPCOYDGRILAVKVTGYVTVHSGDEVELKNLVGTEDLPAVALDADSDFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
AAM11647 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
CAC12806 EGQCRYNQLGVAKVTGYVTVHSGSEVELKNLVGAERPAVAALDVEDSFMMYRSGIYCSQTCSPDRLNHGLAVGYICDGTDYWIVKNSWGLWGEDGY
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P80528 -----|-----
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AAB29986 -----|-----
Q09093 -----|-----
P80532 -----|-----
AAB35021 -----|-----
P80342 -----|-----
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Figure 7. Continued

AAA29137 IEMVVKRGNMCGIASLASVEMVAQEP
 AAF76330 IEMVVKRGNMCGIASLASVEMVAQEP
 AAP49831 IEMARNRGNMCGIASLASLEIGTISV
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 AAA29136 IEMARNRGNMCGIASLASLEMVAREP
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 AAM11647 IEMARNRGNMCGIASLASVAMVAREP
 CAC12806 IEMARNRGNMCGIASLASLEMVAREP
 CAC12805 MRFARNRGNMCAIASVASVEMVEREP
 P80528 -----
 AAK38169 IEMARNRGNMCGIASLASLEMVAREP
 CAC12807 MRFARNRGNMCAIASVASVEMVEREP
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 CAA80444 -----

Figure 7. Continued

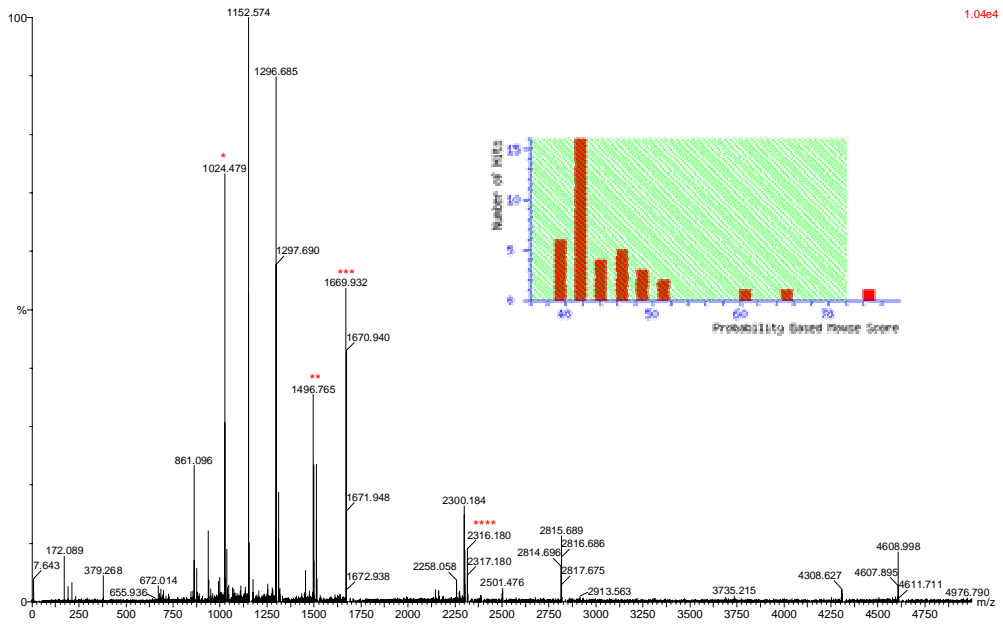


Figure 8. A representative MALDI-ToF PMF from spot 7; top hit *O. aries* Superoxide Dismutase. Red stars correspond to the following peptides *HGGPKDEER, **TMVVHEKPDDLGR, ***AVCVLKGDDGPVQGTIR and ****TMVVHEKPDDLGRGGNEESTK. Shown inset is the MASCOT indication of significance for the hit at P = 5%.

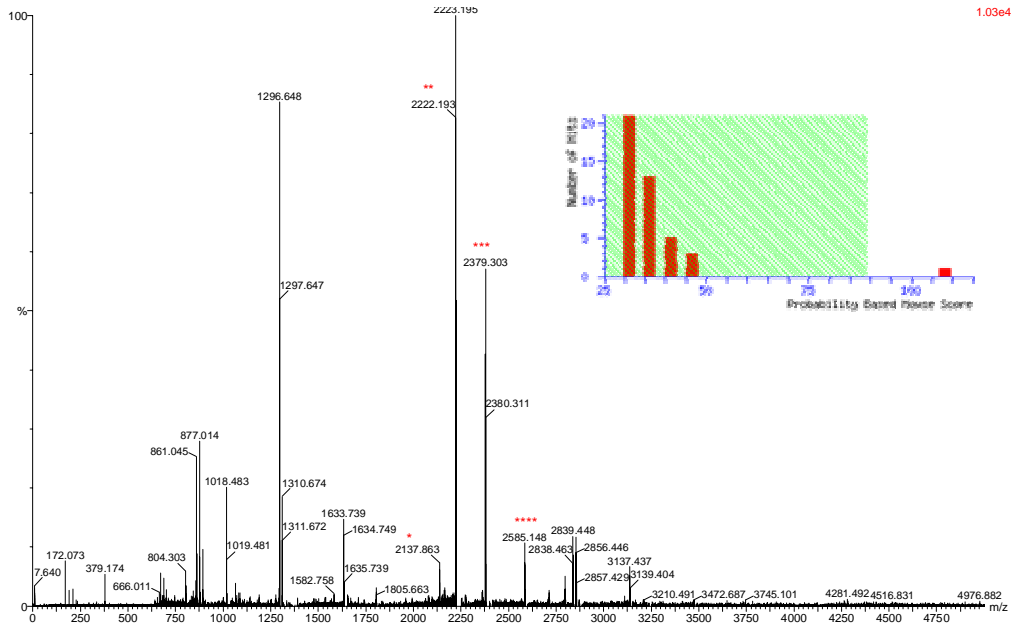


Figure 9. A representative MALDI-ToF PMF from spot 8; top hit *O. aries* Carbonic anhydrase II. Red stars correspond to the following peptides *SHHWGYGEHNGPEHWHK (Acetyl N-term & N-term sequence (m/z 2136.866)), **AVVPDPALKPLALLYEQAASR, ***AVVPDPALKPLALLYEQAASRR (1 missed cleavage (m/z 2378.312) and ****LVQFHFHWGSSDDQGSSEHTVDR (m/z 2584.136). Shown inset is the MASCOT indication of significance for the hit at $P = 0.1\%$.

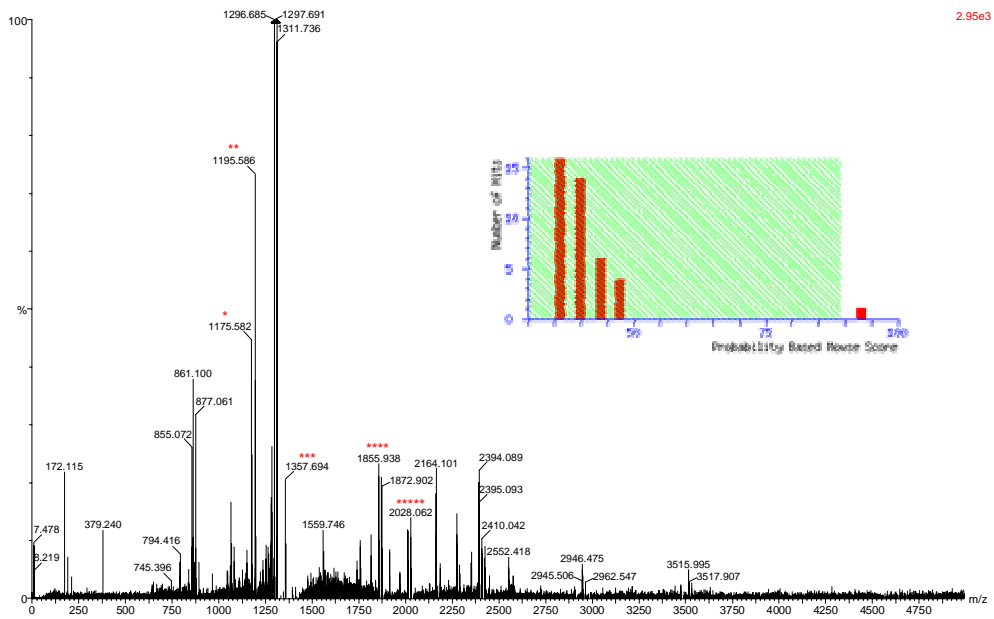


Figure 10. A representative MALDI-ToF PMF from spot 9; top hit *B. taurus* Regucalcin. Red stars correspond to the following peptides *FNDGKVPAGR, **LWVACYNGGR, ***GLLQPEAGGIFK, ****YFAGTMAEETAPAVLER and *****YFAGTMAEETAPAVLERR (Oxidation (M) and 1 Missed Cleavage). Shown inset is the MASCOT indication of significance for the hit at $P = 0.1\%$.


```

Ovis_Blast      -----HAGNKLAMQEFMILPVGASSFRE 23
Bos_Blast      AGAAEKGVPLYRHIADLAGNPILPVPFNVINGGSHAGNKLAMQEFMILPVGASSFRE 180
Bos_PMF        AGAVEKGVPLYRHIADLAGNAEVLVVPFNVINGGSHAGNKLAMQEFMILPVGAEENFRE 180
Fasciola_Blast AGAAEKGLPLYKYIATLAGNKEVIMPVPSFNVINGGSHAGNKLAMQEFMIMPTGASSFTE 179
                *****:*.***. *

Ovis_Blast      AMRIGAEVYHHLKGVIKAKYKDATNVGDEGGFAPNILENNEALELLKTAIQAAGYPDKV 83
Bos_Blast      AMRIGAEVYHHLKGVIKAKYKDATNVGDEGGFAPNILENNEALELLKTAIQAAGYPDKV 240
Bos_PMF        AMRIGAEVYHNLKNVIKEKYKDATNVGDEGGFAPNILENKEALELLKNAIGKAGYSDKV 240
Fasciola_Blast AMKIGSEVYHNLRAVIKSKYGLDACNVGDEGGFAPSIQDNLEGLELLRTRIDKAGYTGKV 239
                **.*:***.*: ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Ovis_Blast      VIGMDVAASEFYRNGKYDLDFKSP-DDP----- 110
Bos_Blast      VIGMDVAASEFYRNGKYDLDFKSP-DDPARHISGEKLGELYKNFIKNYPVVSIEDPFDQD 299
Bos_PMF        VIGMDVAASEFYRSGKYDLDFKSP-DDPSRYITPDELANLYKSFIRDYPVVSIEDPFDQD 299
Fasciola_Blast XIAMDCAASEFYREGKYDLDFKNPKSQASSWITSDAMADVYKMMSTYPIVSIEDPFDQD 299
                *.* * * * * * * * * * * * * * * * * * * * * * * * * * * * *
                ^ ^ ^ ^

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Figure 11. Sequence alignment of enolase entries for confirmation of a host protein rather than of the parasite. Yellow shaded boxed region indicates a peptide hit during PMF analysis conserved between *Ovis aries* sequence and both *Bos taurus* sequences but not in *Fasciola* sequences (blue shaded). Arrows indicate differences in the *Fasciola hepatica* sequence when compared to sequences from hosts. GENBANK accession numbers; Ovis_Blast (AAF60279), Bos_Blast (AAI02989), Bos_PMF (NP_776474) and Fasciola_Blast (AAA57450).

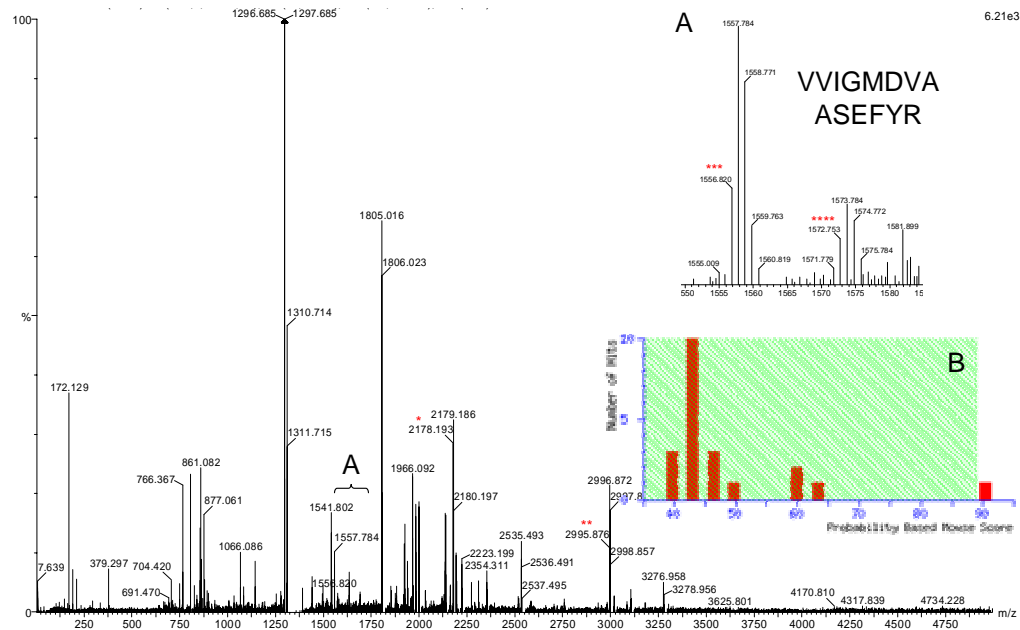


Figure 12. A representative MALDI-TOF PMF from spot 10; top hit *B. taurus* Enolase. Red stars correspond to the following peptides *AGYSDKVIGMDVAASEFYR, **HIADLAGNAEVLVVPFNVINGGSHAGNK ***VIGMDVAASEFYR and ****VIGMDVAASEFYR (1 Met-ox). Shown inset (A) is an enlarged section of the same spectrum showing the location of the unique peptide (***) used to identify this as host enolase (See also figure 10). Shown inset (B) is the MASCOT indication of significance for the hit at P = 0.1%.

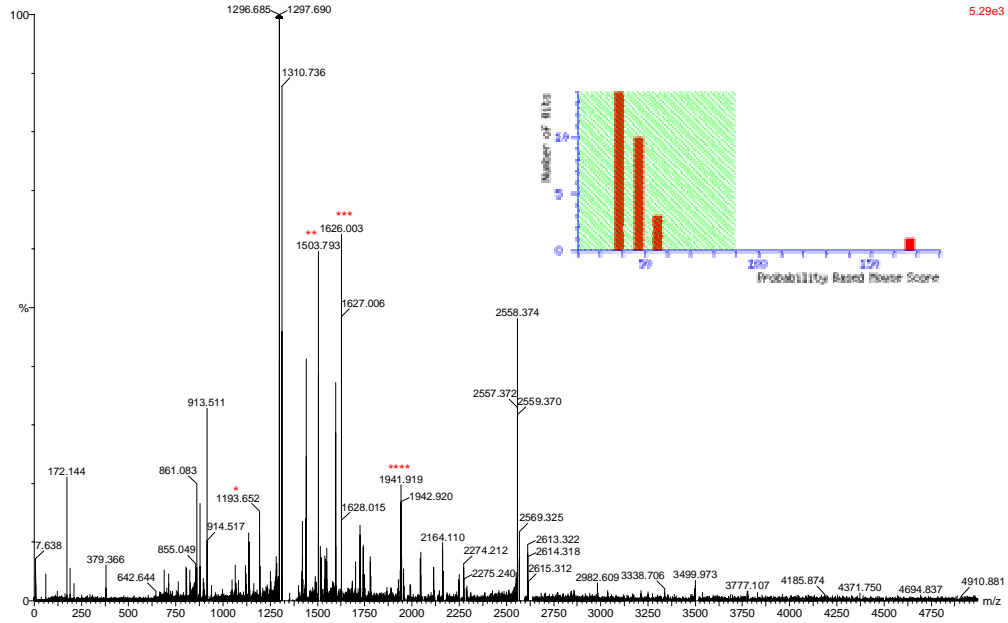


Figure 13. A representative MALDI-ToF PMF from spot 11; top hit *O. aries* Serum albumin precursor. Red stars correspond to the following peptides *DTHKSEIAHR, **HGEYGFQNALIVR, ***KAPQVSTPTLVEISR and ****FTFHADICTLPDTEK. Shown inset is the MASCOT indication of significance for the hit at P = 0.1%.

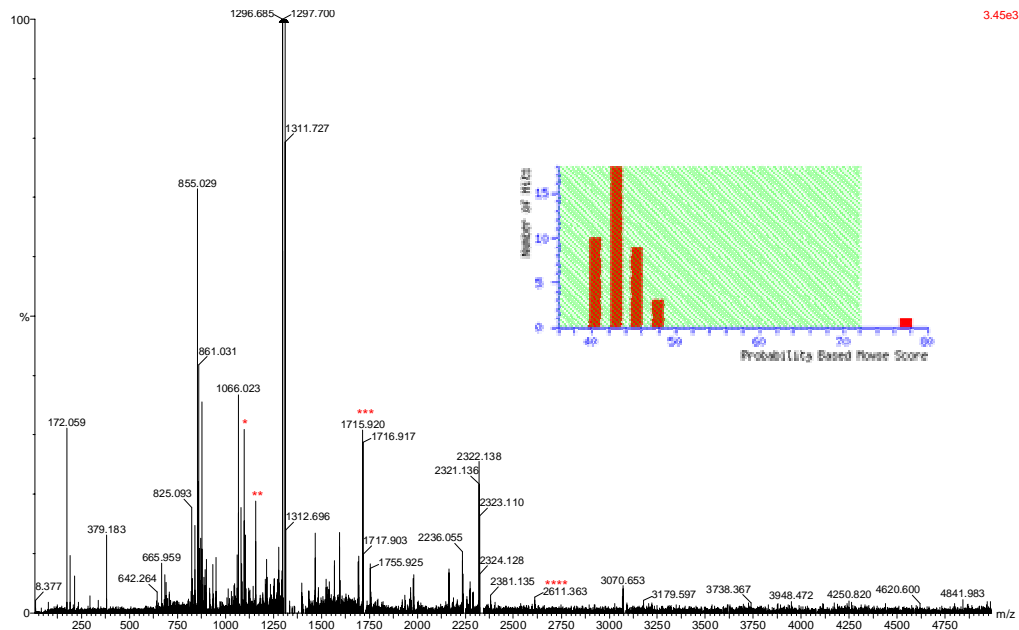


Figure 14. A representative MALDI-ToF PMF from spot 12; top hit *B. taurus* Transferrin. Red stars correspond to the following peptides *YYGTGAFR (m/z 1097.52), **WCAIGHQER (m/z 1156.57), ***LYKELPDPQESIQR and ****MRPAVRLACAVLGLCLADPER (N-Term Sequence). Shown inset is the MASCOT indication of significance for the hit at P = 5%.

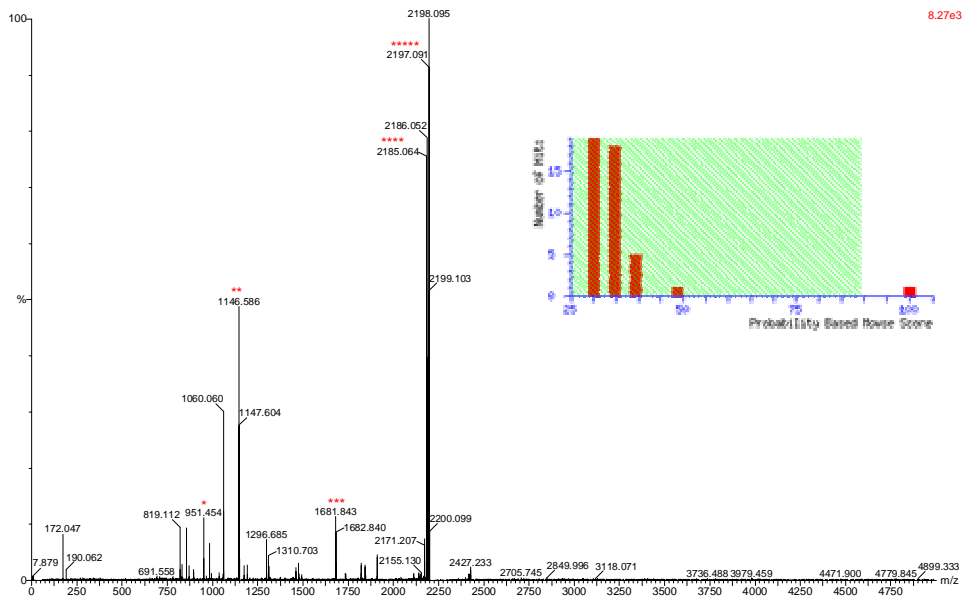


Figure 15. A representative MALDI-ToF PMF from spot 13; top hit *F. hepatica* FABP II. Red stars correspond to the following peptides *ADFGSWK (N-Term sequence), **GPEHTTHIVR, ***NEKPEFTFELEGNK, ****TTTTFTFGEEFKDETFDNR and *****NEKPEFTFELEGNKMTIK (Acetyl N-term and 1 missed cleavage). Shown inset is the MASCOT indication of significance for the hit at P = 0.1%.

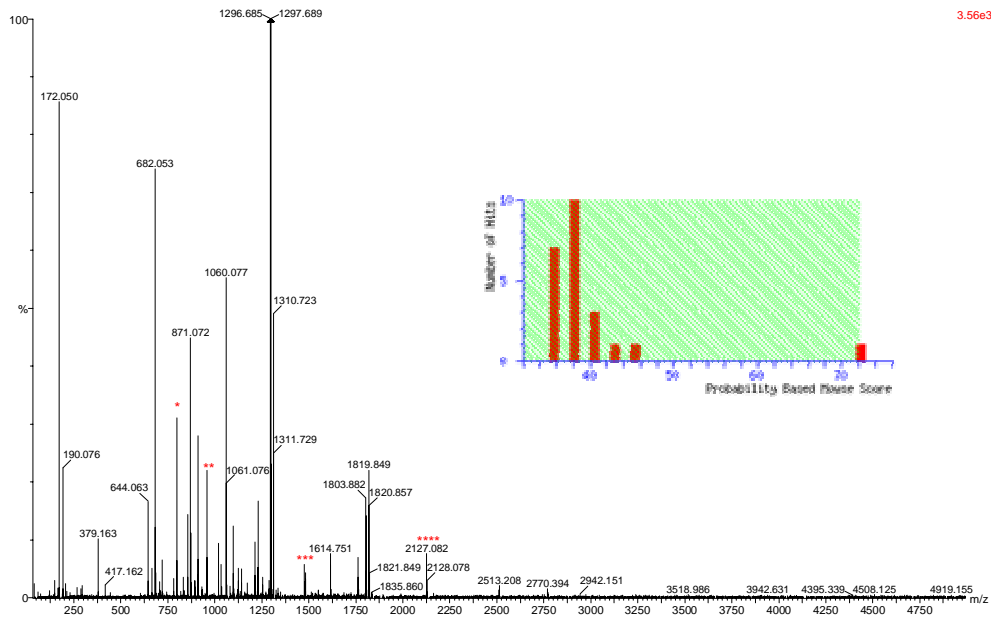


Figure 16. A representative MALDI-ToF PMF from spot 14; top hit *F. hepatica* GST 51. Red stars correspond to the following peptides *GLQQPVR (m/z 797.462), **SRIEDLPK (m/z 957.522), ***YLAPQCLEDFFPK (m/z 1480.670) and *****LLELYGEEYEEHLYGR (m/z 2126.090). Shown inset is the MASCOT indication of significance for the hit at P = 5%.

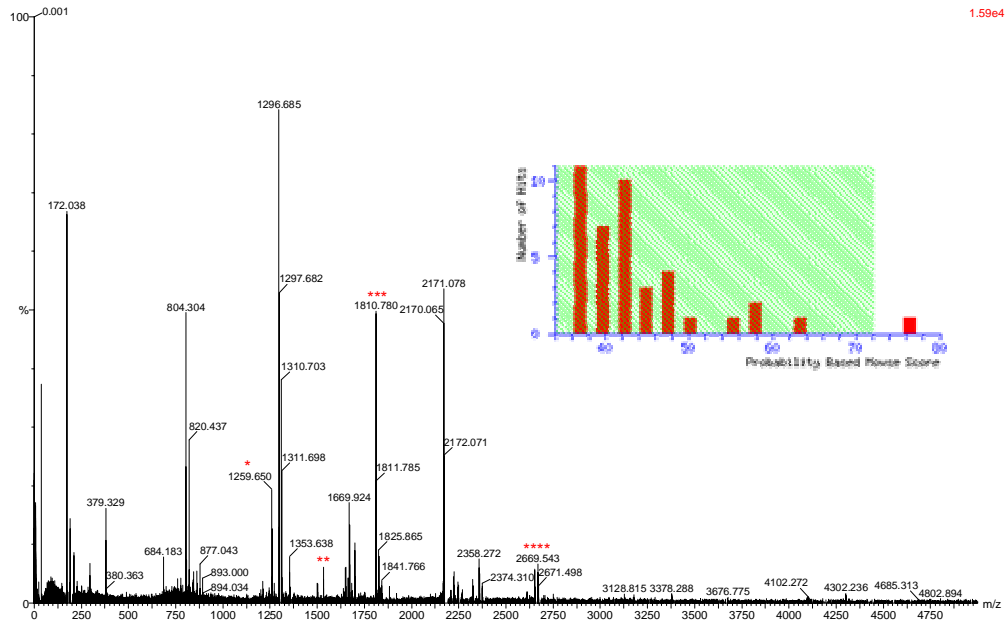


Figure 17. A representative MALDI-ToF PMF from spot 15; top hit *F. hepatica* GAPDH. Red stars correspond to the following peptides *VVDLINHMFR (Oxidation (M)), **VPTADSVVDLTCR (*m/z* 1531.802), ***LISWYDNEFGYSCR (*m/z* 1809.790) and ****VINDKFGIVEGLMTTVHSYTATQK ((Oxidation (M) *m/z* 2668.561). Shown inset is the MASCOT indication of significance for the hit at P = 1%.

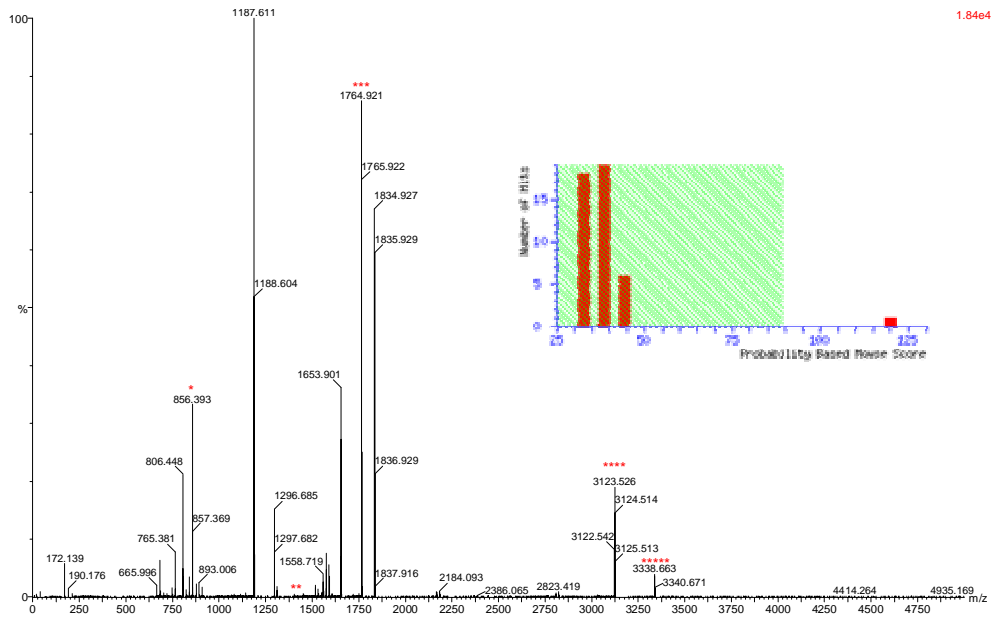


Figure 18. A representative MALDI-ToF PMF from spot 16; top hit *F. hepatica* enolase. Red stars correspond to the following peptides *YAGENFR, **IAMDCAASEFYK (*m/z* 1405.544), ***AAVPSGASTGVHEALELR, ****YGLDACNVGDEGGFAPSIQDNLEGLELLR (*m/z* 3122.542) and ****SKYGLDACNVGDEGGFAPSIQDNLEGLELLR (*m/z* 3337.679). Shown inset is the MASCOT indication of significance for the hit at P = 0.1%.

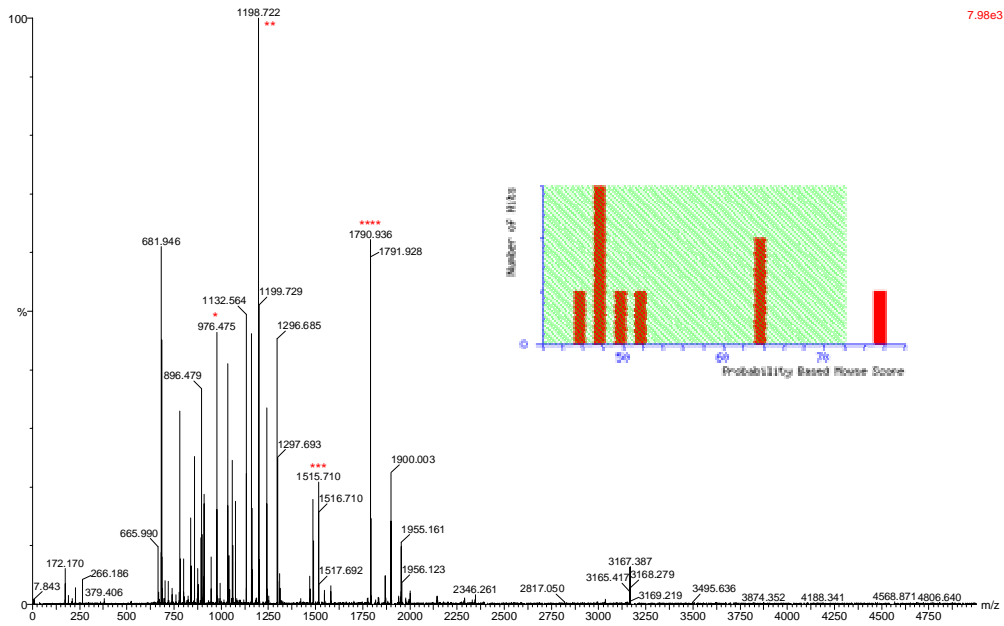


Figure 19. A representative MALDI-ToF PMF from spot 17; top hit *D. dendriticum* actin. Red stars correspond to the following peptides *AGFAGDDAPR, **AVFPSIVGRPR, ***IWHHTFYNELR and ****SYELPDGQVITIGNER. Shown inset is the MASCOT indication of significance for the hit at P = 1%.

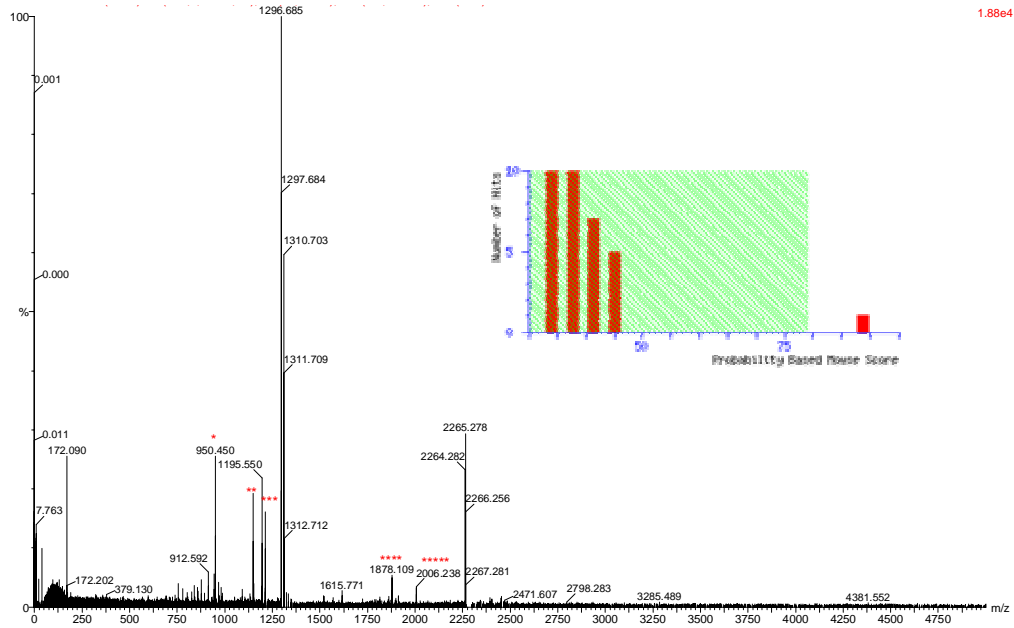


Figure 20. A representative MALDI-ToF PMF from spot 18; top hit *F. hepatica* FABP. Red stars correspond to the following peptides *ANFVGSWK (Acety N-term), **MIATVTVGDVK (m/z 1149.594 Oxidation (M)), ***CPENTTHVVR (m/z 1212.568), ****LITSSKPEITFTLEGNK and *****KLITSSKPEITFTLEGNK (1 missed cleavage). Shown inset is the MASCOT indication of significance for the hit at P = 1%.

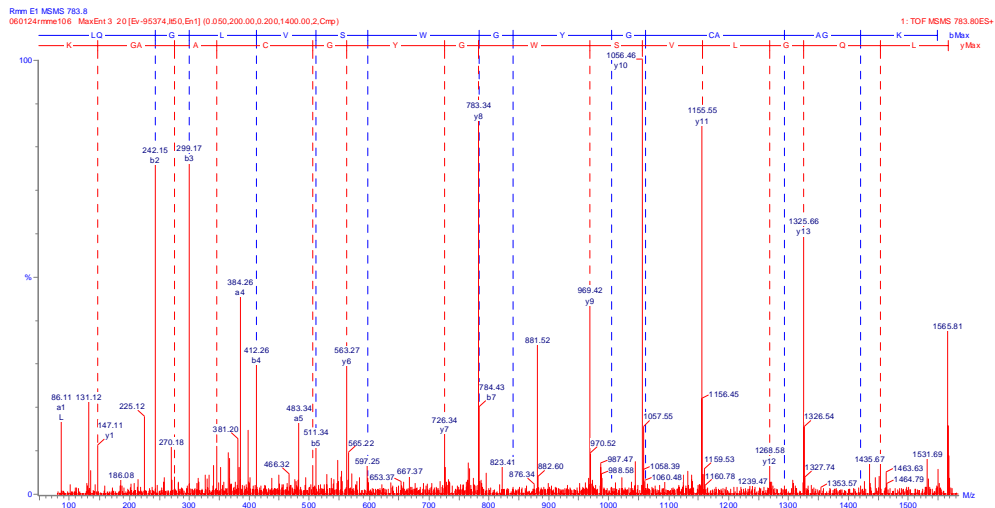


Figure 22. MSMS sequence analysis using peptide sequencer (MassLynx v. 5.0, Micromass, UK) from the fragmentation of a precursor ion m/z 783.8 taken from spots A and B. Interpretation of the y and b ion series provided the peptide sequence LQGVSWGYGCAAGK and corresponded to a peptide from a bovine (*Bos taurus*) trypsin inhibitor complex.

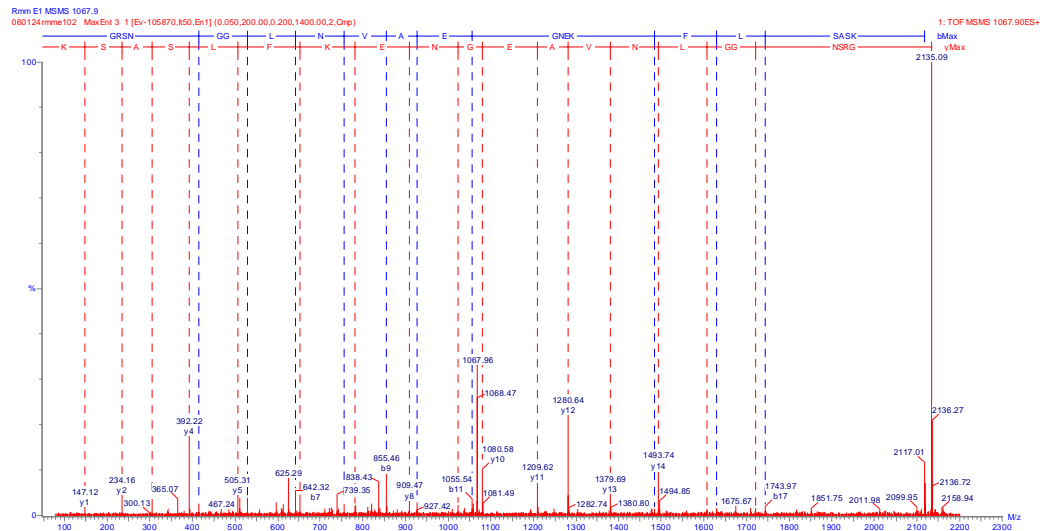


Figure 23. MSMS sequence analysis using peptide sequencer (MassLynx v. 5.0, Micromass, UK) from the fragmentation of a precursor ion m/z 1067.9 taken from spots A and B. Interpretation of the y and b ion series provided the peptide sequence GRSNGGINVAEGNEKFISASK and corresponded to a peptide from a bovine (*Bos taurus*) trypsin inhibitor complex.

Table 3. Peptide sequences derived from MSMS from spots A and B; Identical sequence matches highlighted in red (see figure 24)

Peptide M _r	Precursor Ion Charge State	Sequence
1219.3844	2 ⁺	WVSAAH
1565.5844	2 ⁺	LQGIVSWG YGCAAGK
2133.7844	2 ⁺	GRSNGGIN V AEGNEK FISASK

Chain A, Trypsin Inhibitor Complex (Bpo) Score 52.0 **e-value 4e-06**

1EB2_A *B. taurus* Chain A, Trypsin Inhibitor Complex (Bpo)

IVGGYTCGANTVPYQVSLNSGYHFCGGSLNSQ**WVSAAH**CYKSGIQVRLGEDN**IN**V**EGNEQ**FISASK
 SIVHPSYNSNTLNNDIMLIKLSAASLNSRVASISLPTSCASAGTQCLISGWGNTKSSGTSYPDLKCLKA
 PILSDSSCKSAYPGQITSNMFCAGYLEGGKDCQGDGGGPVVCSGKLQ**IVSWG**S**GCAQ**KNKPGVYT
 KVC NYVSWIKQTIASN

Figure 24. Top result from BLAST analysis using the 3 peptides above from spots A and B (Table 3); 29 out of a total of 223 amino acids were identified by MSMS analysis therefore giving 13% sequence coverage.

Table 4. Progenesis expression data for spots 13; FABP type II (*F. hepatica*).
 *Significance set at >2 fold increase/decrease

Spot Number (Progenesis Spot Number)	ES Culture (h)	Normalised Volume	Difference (Versus 2h)
13 (563)	2	39.238	-
13 (563)	4	47.744	1.217
13 (563)	8	183.564	3.845*
13 (563)	16	379.208	7.943*
13 (562)	2	80.547	-
13 (562)	4	82.147	1.02
13 (562)	8	203.961	2.812*
13 (562)	16	133.332	1.623

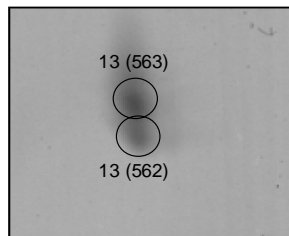


Table 5. Progenesis expression data for spots 16 & 17; Enolase (*F. hepatica*) and Actin (*D. dendriticum*). *Significance set at >2 fold increase/decrease

Spot Number (Progenesis Spot Number)	ES Culture (h)	Normalised Volume	Difference (Versus 2h)
16 (410)	2	51.645	-
16 (410)	4	80.524	1.559
16 (410)	8	135.541	2.624*
16 (410)	16	122.914	2.380*
17 (403)	2	35.643	-
17 (403)	4	43.645	1.225
17 (403)	8	195.611	5.488*
17 (403)	16	129.674	3.638*

