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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 Morphew, Russell M.; Wright, Hazel A.; LaCourse, E. James; Woods, Debra J.; Brophy, Peter M.

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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

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



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 "ASASFSEQQLVDCTR, "VTGYYTVHSGDEIELK, "**LTHAVLAVGYGSQDGTDYWIVK 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, **TSISFSEQQLVDCSR and ***FGLETESSYPYR. 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 "NDDUWHQWK (N-terminal of Sequence in Propeptide (*m*/z Submitted 1241.53, *m*/z Matched 1241.56)), **VTGYYTVHSGSEVELK 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, ***VTGYTVHSQSEVELK and ***QFGLETESSYPYTAVEGQCR. 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	MRLVILTLLIVGVFASNEDIWHOWKRIYNKEYKGAEEDHRRNIWEONVKHIOEHNLRHDIGLVTYKLGLNOFTEMTFEEFKAKYLTEMFRASELLSHGIP
AAF76330	MRLVILTLLIVGVFASNEDLWHQWKRIYNKEYNGAEEDHRRNIWEQNVKHIQEHNLRHDLGLVTYKLGLNQFTDMTFEEFKAKYLTEMFRASELLSHGIP
AAP49831	MWFFVLAVLTVGVLGSNEDLWHQWKRMYNNEYNGAELQHRRNIWEENVKHIQEHNLRHDLGLVTYTLGLNQFTDMTFEEFKAKYLTEMSRASEILSHGVP
BAA23743	MRLFILAVLTVGVLGSNDDLWHQWKRMYNKEYNGAVCEHRRNIWEENVKHIQEHNLRHDLGLVTYTLGLNQLTDMTFEEFKAKYLTEMFRASCILSHGIP
AAA29136	MRLFILAVLTVGVLGSNEDLWHOWKRMYNKEYNGADLOHRRNIWEKNVKHIOEHNLRHDLGLVTYTLGLNOFTDMTFEEFKAKYLTEMSRASEILSHGVP
AAR99518	MRLFILAVLTVGVLGSNEDLWHOWKRMYNKEYNGADLEHRRNIWEENVKHIOEHNLRHDLGLVTYTLGLNOFTDMTFEEFKAKYLTEMSRASEILSHGVP
AAB41670	MRLFILAVLTVGVLGSNEDLWHQWKRMYNKEYNGAELQHRRNIWEKNVKHIQEHNLRHDLGLVTYTLGLNQFTDMTFEEFKAKYLTEMSRASCILSHGVP
AAC47721	MRCFVLAVLTVGVYASNEDLWHQWKRIYNKEYNGAELEHRRNIWCKNVKHIQEHNLRHDLGLVTYKLGLNQFTDLTFEEFKAKYLIEIFRSSELLSRGIP
Q24940	MRLFILAVLTVGVLGSNEDLWHQWKRMYNKEYNGAELQHRRNIWEKNVKHIQEHNLRHDLGLVTYTLGLNQFTDMTFEEFKAKYLTEMSRASCILSHGVP
AAT76664	MRLFILAVLTVGVLGSNEDLWHQWKRMYNKEYNGAELQHRRNIWEKNVKHIQEHNLRHDLGLVTYTLGLNQYTDLTFEEFKAKYLTEMFRASCILSHGIP
CAA80446	MRFFVLAVLTVGVFASNDDLWHQWKRIYNKEYNGADDEHRRNIWGKNVKHIQEHNLRHGLGLVTYKLGLNQFTDLTFEEFKAKYLIEIFRSSELLSRGIP
AAM11647	SNDDLWHQWKRMYNKEYNGADEQHRRNIWEKNVKHIQEHNLRHDLGLVTYTLGLNQFTDMTFEEFKAKYLTEMSRASCILSHGVP
CAC12806	SNDDLWHQWKRMYNKEYNGADDEHRRNIWEENVKHIQEHNLRHDLGLVTYTLGLNQFTDMTFEEFKAKYLTEMPRASDILSHGIP
CAC12805	SNEVSWHEWKRMYNKEYNGALEEHRRNIWGKNVKHIEEHNLRHDRGLVTYKLGLNQFTDFTFEEFCAKYLMEMSPVSESLSDGVS
P80528	
AAK38169	
CAC12807	WHEWKRMYNKEYNGADDEHRRNIWEQNAKHIEEHNIRHDRGLVTYKLGLNQFTDITFEEFKAKYIMEMSPVSESLSDGIS
AAR99519	MSRASDILSHGIP
AAB29986	
Q09093	
P80532	
AAB35021	
P80342	
CAA80450	
CAA80448	
CAA80447	
CAA80445	
CAA80444	

Figure 7. Sequence alignment of all *F. hepatica* cathepsin L sequences within Genbank (<u>http://www.ncbi.nlm.nih.gov</u>); 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.

	I
AAA29137	YKANKEAVEDRIDWRESGYVTEVKDQGCCGSCWAFSTTGAMECQYMKNEKTSISFSEQQLVDCSGPFGNYGCNGGLMENAYEYLKRFGLETESSYPYRAV
AAF76330	YKANKRAVPDRIDWRESGYVTEVKDQGGCGSCWAFSTTGAMECQYMKNQRTSISFSEQQLVDCSRDFGNYGCNGGLMENAYEYLKRFGLETESSYPYRAV
AAP49831	YETNNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGTMEGQYMKNERTSISFSEQQLVDCSGPWGNNGCSGGLMENAYÇYLKQFGLETESSYPYTAV
BAA23743	YEANNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGIMEQQYMKNERTSISFSEQQLVDCSGPWGNYGCMGGLMENAYEYLKQFGLETESSYPYTAV
AAA29136	YEANNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGTMEGQYMKNERTSISFSEQQLVDCSGPWGNNGCSGGLMENAYÇYLKQFGLETESSYPYTAV
AAR99518	YETNNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGTMEGQYMKNERTSISFSEQQLVDCSGPWGNNGCSGGLMENAYQYLKQFGLETESSYPYTAV
AAB41670	YEANNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGTMEGQYMKNERTSISFSEQQLVDCSRPWGNNGCGGGLMENAYÇYLKQFGLETESSYPYTAV
AAC47721	FKANKLAVFESIDWREYYYVTEVK <mark>NQGQCCSCWAFSTTGAVECQFR</mark> KNERASASFSEQQLVDCPRDLGNYGCGGGYMENAYEYLKHNGLETESYYPYQAV
Q24940	YEANNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGIMECQYMKNERTSISFSEQQLVDCSGPWGNNGCSGGLMENAYCYLKQFGLETESSYPYTAV
AAT76664	YEANNRAVPDKIDWRESGYVTGVKDQGNCGSCWAFSTTGTIECQYMKNERISISFSEQQLVDCSGPWGNNGCGGGLMENAYEYLKQFGLETESSYPYTAV
CAA80446	YKANKIAVFESIDWREYYYVTEVK <mark>DQGQCCSCWAFSTTGAVECQFR</mark> KNERASASFSEQQLVDCTRDFCNYGCGGGYMENAYEYLKHNGLETESYYPYQAV
AAM11647	YEANNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGIMECQYMKNERISISFSEQQLVDCSGPWGNNGCSGGLMENAYCYLKQFGLETESSYPYTAV
CAC12806	YEANNPAVPDKIDWRESGYVTGVKDQGNCGSCWAFSTTGTMEGQYMKNEKTSISFSEQQLVDCSGPWGNNGCSGGLMENAYEYLKRFGLETESSYPYRAV
CAC12805	YEAEGNDVEASIDWREYGYVTEVKDQGQCGSCWAFSAVGAIECQYVKKFQNÇTIFSEQQLVDCTRRFGNHGCGGGWMENAYKYLKNSGLETASYYPYQAV
P80528	
AAK38169	YEANNRAVPDKIDWRESGYVTEVKDQGNCGSCWAFSTTGIMECQYMKNERTSISFSEQQLVDCSGPWGNNGCSGGLMENAYQYLKQFGLETESSYPYTAV
CAC12807	YEAEGROVFASIDWRCYGYVTEVRDQGQCGSCWAFSPVGAIECQYVRRFQNCTLFSEQQLVDCTRRFGNHGCGGGMMENAYRYLRNSGLETASDYPYQGW
AAR99519	YEANNRAVPDRIDWRESGYVIGVRDQGNCGSCWAFSTIGIMEGQYMKNERTSISPSEQQLVDCSGPWGNNGCSGGLMENAYQYLKQFGLEIESSYPYIAV
AAB29986	AVPDRIDPRESGYVTGVRDQ
Q09093	AVPDKIDPRESGYVIGVKDQ
P80532	DVPASIDWREYGYVIEVKD
AAB35021	DVPASIDWRBYGYVTEVKD
P80342	AVPDKIDKRESGYV
CAA80450	QQCGTCWAPSTIGTMECQTMRKCRISTSPSDEQLVDCSRPWGNRGCGGGLMENAYCYLKCPGLETESSYPTTAV
CAA80448	
CAA80447	QGQCGRCWAFSTIGATECQTMKNCRISISFSEQULVDCSRDFGNYGCNGGLMENAYFYLKRFGLETESSYPYRAV
CAA80445	
CAA80444	UGQUGWCWAFSTIGALEGQIMKSQKINISFSEQQLVDCSGDFGNHGCSGGLMEKAYEYLRHFGLETESSYSYRAD
	1

Figure 7. Continued

AAA29137	EGQCRYNEQLGVAKVTGYYTVHSGDEVELQNLVGCRRPAAVALDVESDFMMYRSGIYCSQTCSPDRLNHGVLAVGYGICDGTDYWIVKNSWGTWWGEDGY ⁰
AAF76330	EGQCRYNEQLGVAKVTGYYTVHSGDEVELQNLVGAEGPAAVALDVESDFMMYRSGIYCSQTCSPDRLNHGVLAVGYGICDGTDYWIVKNSWGTWWGEDGY
AAP49831	EGQCRYNKQLGVAKVTGYYTVFSGSEVEIKNLVGAEGFAAVAVDVESDFMMYRSGIYCSQTCSPLRVNHAVLAVGYGTCGGTDYWIVKNSWGLSWGERGY
BAA23743	EGQCRYNFQLGVAKVTDYYTVHSGSEVELKNLVGAEGFAAVAVDVESDFMMYSCGIYCSRTCSSLRVNHAVLAVGYGTCGGTDYWIVKNSWGSSWGER-Y
AAA29136	EGQCRYNKQLGVAKVTGYYTVHSGSEVELKNLVGARRPAAVAVDVESDFMMYRSGIYÇSQTCSPLRVNHAVLAVGYGTÇGGTDYWIVKNSWGTYWGERGY
AAR99518	EGQCRYNEQLGVAKVTGYYTVHSGSEVEIKNLVGSEGFAAVAVDVESDFMMYRSGIYCSQTCSPLSVNHAVLAVGYGTCGGTDYWIVKNSWGLSWGERGY
AAB41670	CGQCRYNKQLGVAKVTGYYTVÇSGSEVELKNLIGSEGFSAVAVDVESDFMMYRSGIYÇSQTCSPLRVNHAVLAVGYGTÇGGTDYWIVKNSWGLSWGERGY
AAC47721	EGPCQYDGRLAYAKVTGYYTVHSGDEIEIKNLVGTEGFAAVALDADSDFMMYQSGIYCSQTCLPDRLTHAVLAVGYGSCDGTDYWIVKNSWGTWWGEDGY
Q24940	EGQCRYNKQLGVAKVTGYYTVHSGSEVELKNLVGARRPAAVAVDVESDFMMYRSGIYÇSQTCSPLRVNHAVLAVGYGTÇGGTDYWIVKNSWGTYWGERGY
AAT76664	EGQCRHSKQLGVAKVTGYYTVHSGSEVELKNLVGAERPAAVAVDVESDFMMYRSGIYÇSQTCSPLSVNHAVLAVGYGTÇGGTDYWIVKNSWGLSWGERGY
CAA80446	EGPCQYDGRLAYAKVTGYYTVHSGDEIEIKNLVGTEDLPAVALDADSDFMMYQSGIYCSQTCLPDRLTHAVLAVGYGSCDGTDYWIVKNSWGTWWGEDGY
AAM11647	EGQCRYNKQLGVAKVTGYYTVHSGSEVELKNLVGAEGPAAVAVDVESDFMMYRSGIYÇSQTCSPLRVNHAVLAVGYGTÇDGTDYWIVK <mark>NSWGSYWGER</mark> GY
CAC12806	EGQCRYNEQLGVAKVTGYYTVHSGSEVELKNLVGSEGPAAIAVEAESDFMMYRSGIYÇSQTCLPFALNHAVLAVGYGTÇDGTDYWIVKNSWGLSWGERGY
CAC12805	EYQCQYRKELGVAKVTGAYTVHSGDEMKIMFMVGREGPAAVAVDACSDFYMYESGIFCSQTCTSRSVTHAVLAVGYGTESGTDYWILKNSWGKWWGEDGY
P80528	
AAK38169	EGQCRYNRQLGVAKVTGYYTVHSGSEVELKNLVGSRRPAAIAVDVESDFMMYRSGIYÇSQTCLPFALNHAVLAVGYGTÇDGTDYWIVKNSWGLSWGERGY
CAC12807	EYQCQYRKELGVAKVTGAYTVHSGDEMKIMEMVRKKGPAAAAVDAQEDFYMYESGIFQSQYCSSRRVTHAVLAVGHGTESGTDYWILKNSWGKWWGEDGY
AAR99519	EGQCRYNRQLGVAKVTGYYTVHSGSEVELKNLVGSEGPAAIAVDVESDFMMYRSGIYÇSQTCLPFALNHAVLAVGYGTÇGGTDYWIVKNSWGLSWGERGY
AAB29986	
Q09093	
P80532	
AAB35021	
P80342	
CAA80450	EGQCRYNEQLGVAKVTGYYTVHSGSEVELKNLVGSEGPARSPVDVESDFMMYRSG1YCSGTCLPFALNHAVLAVGYGTCDGTDYNIVKNSW
CAA80448	LNRCRANRTEGIVKVKSYTVLKNESETHSRSWSGTRGPVAVGTHADLGFÇFYSBGTYVSSTCSSWPANHGVLVVGYGAEAANSPYWIVKNIW
CAA80447	EGOCKTREDEGVAKVTGTTTVHSGDEVELQREVGAGREPAAVALDVESDFMMYRSGTYCSTCSPDRLNHGVLAVGYGTCDGTDVHVVKNTW
CAA80445	EGPCQTIC:RLAYAVVIGTTIVHSQDBLEEKRIVGIBDEPAVALDADSDFMMYQSGTYCSQTCLPDRETHAVLAVGYQSQDCTDYWIVKNSW
CAA80444	EGPCQIDKQEGVAQVSGIFIVHSQDEVALKNEIGVEGPAAVALDVNIDFMMYRSGIYQDEICSSRYLNHAVLAVGYGTEDCIDYWIVKNIW

Figure 7. Continued

AAA29137	IRMVRKRGNMCGIASLASVFMVAQFP
AAF76330	IRMVRKRGNMCGIASLASVFMVAQFP
AAP49831	IRMARNRGNMCGIASLASLFIGTISV
BAA23743	IRMVRNRGNMCGIASLASLFMVARFP
AAA29136	IRMARNRGNMCGIASLASLFMVARFP
AAR99518	IRMVRNRGNMCGIASLASLFMVARFP
AAB41670	IRMVRNRGNMCGIASLASLFMVARFP
AAC47721	IRFARNRGNMCGIASLASVFMVARFP
Q24940	IRMARNRGNMCGIASLASLFMVARFP
AAT76664	IRMVRNRGNMCGIASLASLFMVARFP
CAA80446	IRFARNRGNMCGIASLASVFMVARFP
AAM11647	IRMARNRGNMCGIASLASVAMVARFP
CAC12806	IRMARNRGNMCGIASLASLFMVARFP
CAC12805	MRFARNRGNMCAIASVASVFMVERFP
P80528	
AAK38169	IRMARNRGNMCGIASLASLFMVARFP
CAC12807	MRFARNRGNMCAIASVASVFMVERFP
AAR99519	IRMARNRGNMCGIASLASLFMVARFP
AAB29986	
Q09093	
P80532	
AAB35021	
P80342	
CAA80450	
CAA80448	
CAA80447	
CAA80445	
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, ***AVCVLKGDGPVQGTIR 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 ****LVQFHFHWGSSDDQGSEHTVDR (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 *FNDGKVDPAGR, **LWVACYNGGR, ***GLLQQPEAGGIFK, ****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	AGAAEKGVPLYRHIADLAGNPELILPVPAFNVINGGSHAGNKLAMQEFMILPVGASSFRE	180
Bos PMF	AGAVEKGVPLYRHIADLAGNAEVILPVPAFNVINGGSHAGNKLAMOEFMILPVGAENFRE	180
Fasciola Blast	AGAAEKGLPLYKYIATLAGNKEVIMPVPSFNVINGGSHAGNKLAMOEFMIMPTGASSFTE	179
—	***************************************	
Ovis Blast	AMR I GAEVYHHLKGV I KAKYGKDATNVGDEGGFAPN I LENNEALELLKTA I QAAGYPDK	83
Bos Blast	AMRIGAEVYHHLKGVIKAKYGKDATNVGDEGGFAPNILENNEALELLKTAIQAAGYPDK <mark>V</mark>	240
Bos PMF	AMR I GAEVYHNLKNVI KEKYGKDATNVGDEGGFAPN I LENKEALELLKNA I GKAGYSDKV	240
Fasciola Blast	AMKIGSEVYHNLRAVIKSKYGLDACNVGDEGGFAPSIODNLEGLELLRTAIDKAGYTGKV	239
	::****:*: *** *** ** **************	
Ovis Blast	VIGMDVAASEFYRNGKYDLDFKSP-DDP	110
Bos Blast	VIGMDVAASEFYRNGKYDLDFKSP-DDPARHISGEKLGELYKNFIKNYPVVSIEDPFDOD	299
Bos PMF	VIGMDVAASEFYRSGKYDLDFKSP-DDPSRYITPDELANLYKSFIRDYPVVSIEDPFDOD	299
Fasciola Blast	XIAMDCAASEFYKEGKYDLDFKNPKSQASSWITSDAMADVYKKMMSTYPIVSIEDPFDOD	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 *AGYSDKVVIGMDVAASEFYR, *HIADLAGNAEVILPVPAFNVINGGSHAGNK ***VVIGMDVAASEFYR and ****VVIGMDVAASEFYR (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 ****FFTFHADICTLPDTEK. 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 'YYGYTGAFR (m/z 1097.52), 'WCAIGHQER (m/z 1156.57), ''LYKELPDPQESIQR and '''MRPAVRALLACAVLGLCLADPER (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 *ADFVGSWK (N-Term sequence), **GPEHTTHIVR, ***NEKPEFTFELEGNK, ***TTTFTFGEEFKDETFDNR 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), **YLAPQCLEDFPK (m/z 1480.670) and ****LLLEYLGEEYEEHLYGR (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)), **VPTADVSVVDLTCR (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), ***AVPSGASTGVHEALELR, ***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, ***IVHTFYNELR 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 (Acetyl 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%.

Accession Number of Top Hit	E value	Description	Species
CAA80446	41	Androgen induced inhibitor of proliferation	Homo sapiens
AAC47721	15	ENSANGP00000014868 [Anopheles gambiae str. PEST]	Anopheles gambiae
AAF76330	1.7	Hypothetical protein CBG11190	Caenorhabditis elegans
AAK38169	16	Ribosomal protein S24	Marsupenaeus japonicus
AAF44678	24	Unknown	Schistosoma japonicum

ENSANGP00000011628

Mitochondrial malate dehydrogenase precursor

PREDICTED: similar to putative alcohol dehydrogenase

PREDICTED: similar to tryptase 5

Carnitine Palmitoyl Transferase family member (cpt-2)

Nudix-type motif 6 isoform b

Chain E, Crystal Structure Of Human Rhoa In Complex With DhPH Fragment Of Pdzrhogef

Unnamed protein product

Serum amyloid A2

Vitellogenin

Green fluorescent protein-like protein

All Top 20 Hits Actin

T cell receptor alpha chain

Accession Number CAH73160 XP_319000 CAE65999 AAM18049

AAW 26914

EAA00852

NP_005909

XP_623818

XP_795734

NP_502096

NP_932158

1XCG_E

CAG06814

NP_110381

BAB69831

AAK71343

-

AAB47369

Anopheles gambiae

Homo sapiens

Apis mellifera

Strongylocentrotus purpuratus

Caenorhabditis elegans

Homo sapiens

Homo sapiens

Tetraodon nigroviridis

Homo sapiens

Macrobrachium rosenbergii

Condylactis gigantea

Homo sapiens

Table 2. PMF top matches for a highest ranked hit to a non-homologous protein

AAM11647

P09670

P00922

NP_776382

NP_776474

P14639

NP_803450

Q7M4G1

AAB28746

AAG23287

A53665

AAA82603

CAB65015

1.1

0.3

22

11

11

1.1

13

19

30

0.089

28

27



Figure 21. MSMS sequence analysis using peptide sequencer (MassLynx v. 5.0, Micromass, UK) from the fragmentation of a precursor ion m/z 610.7 taken from spots A and B. Interpretation of the y and b ion series provided the peptide sequence WVVSAAH and matched exactly to a peptide from a bovine (*Bos taurus*) trypsin inhibitor complex.



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 LQGIVSWGYGCAAGK 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+	WVVSAAH
1565.5844	2+	LQG <mark>IVSWG</mark> YGCAAGK
2133.7844	2+	GRSNGGINVAEGNEKFISASK

Chain A, Trypsin Inhibitor Complex (Bpo) Score 52.0 e-value 4e-06				
1EB2_A	B. taurus	Chain A, Trypsin Inhibitor Complex (Bpo)		
IVGGYTCGANTVPYQ	SLNSGYHFCGG			

IVGGYTCGANTVPYQVSLNSGYHFCGGSLINSQ**WVVSAAH**CYKSGIQVRLGEDNINVVEGNEQFISASK SIVHPSYNSNTLNNDIMLIKLKSAASLNSRVASISLPTSCASAGTQCLISGWGNTKSSGTSYPDVLKCLKA PILSDSSCKSAYPGQITSNMFCAGYLEGGKDSCQGDSGGPVVCSGKLQGIVSWGSGCAQKNKPGVYT 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.

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 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)
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*

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

