

Mascot introduction and application for protein identification

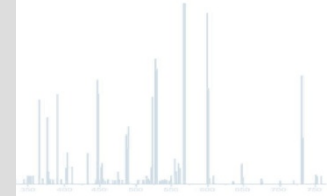
講員: 蔡沛倫
鎂陞科技股份有限公司 **Mass Solutions Technology**

時間: 2011年07月21日

地點: 台灣大學

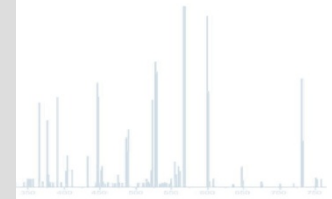


蛋白質資料庫搜尋鑑定 (Mascot)



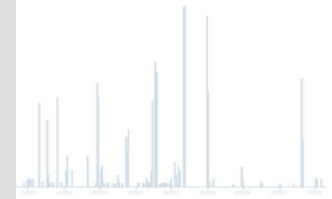
- Mascot蛋白質鑑定的技術原理介紹
- 各項使用參數的意義及建議設定值
- 鑑定分析結果報告呈現
- 新版報告呈現方式

What is MASCOT ?



- MASCOT是一套利用質譜的圖譜與資料庫序列進行比對來鑑定蛋白質的軟體。
- MASCOT提供的比對方式有以下三大類：
 1. Peptide mass fingerprint
 2. Sequence Query
 3. MS/MS Ion search
- MASCOT預設可使用的資料庫如下：
 1. MSDB
 2. NCBI nr
 3. SwissProt
 4. dbEST (nucleic acid database)MASCOT提供使用者自建database比對的功能，方便使用者進行 recombinant protein searching。

Database Example



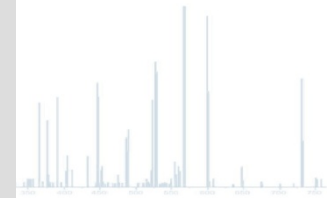
```
>gi|386828|gb|AAA59172.1| insulin [Homo sapiens]  
MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPK  
TRREAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLE  
NYCN
```

```
>gi|1351907|sp|P02769|ALBU_BOVIN Serum albumin precursor (Allergen Bos d 6)  
(BSA)  
MKWVTFISLLLLFSSAYSRGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYL  
QQCPFDEHVKLVLNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYG  
DMADCCEKQEPERNECFLSHKDDSPDLPKLKPDPNTLCDEFKADEKKFWGKYL  
YEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLPKIETMREKVLASS  
ARQRLRCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVTDLTKEVHKECCH  
GDLLECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCIAEVEKDAIPENLP  
PLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEYEATL  
EECCAADDPHACYSTVFDKLVDEPQNLIKQNCQDFEKLGEYGFQNALIVRY  
TRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHE  
KTPVSEKVTKCCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTE  
KQIKKQTALVELLKHKPKATEEQLKTMENFVAFVDKCCAADDKEACFAVEGPK  
LVVSTQTALA
```

MASCOT

網頁免費版: http://www.matrixscience.com/search_form_select.html

In House版: http://localhost/search_form_select.html



Mascot Search

Mascot Help
Mascot Overview
Search parameter reference
Data file format
Scoring algorithm
Results format
Results Interpretation
Error tolerant search
Decoy database search
Quantitation
User Meeting Presentations
2007
More Help
Help Topic Index
Unimod
Useful Links

- **Peptide Mass Fingerprint:** The experimental data are a list of peptide mass values from an enzymatic digest of a protein.
 - [Example of results report](#)
 - [More information](#)
- **Sequence Query:** One or more peptide mass values associated with information such as partial or ambiguous sequence strings, amino acid composition information, MS/MS fragment ion masses, etc. A super-set of a sequence tag query.
 - [Example of results report](#)
 - [More information](#)
- **MS/MS Ion Search:** Identification based on raw MS/MS data from one or more peptides.
 - [Example of results report](#)
 - [More information](#)

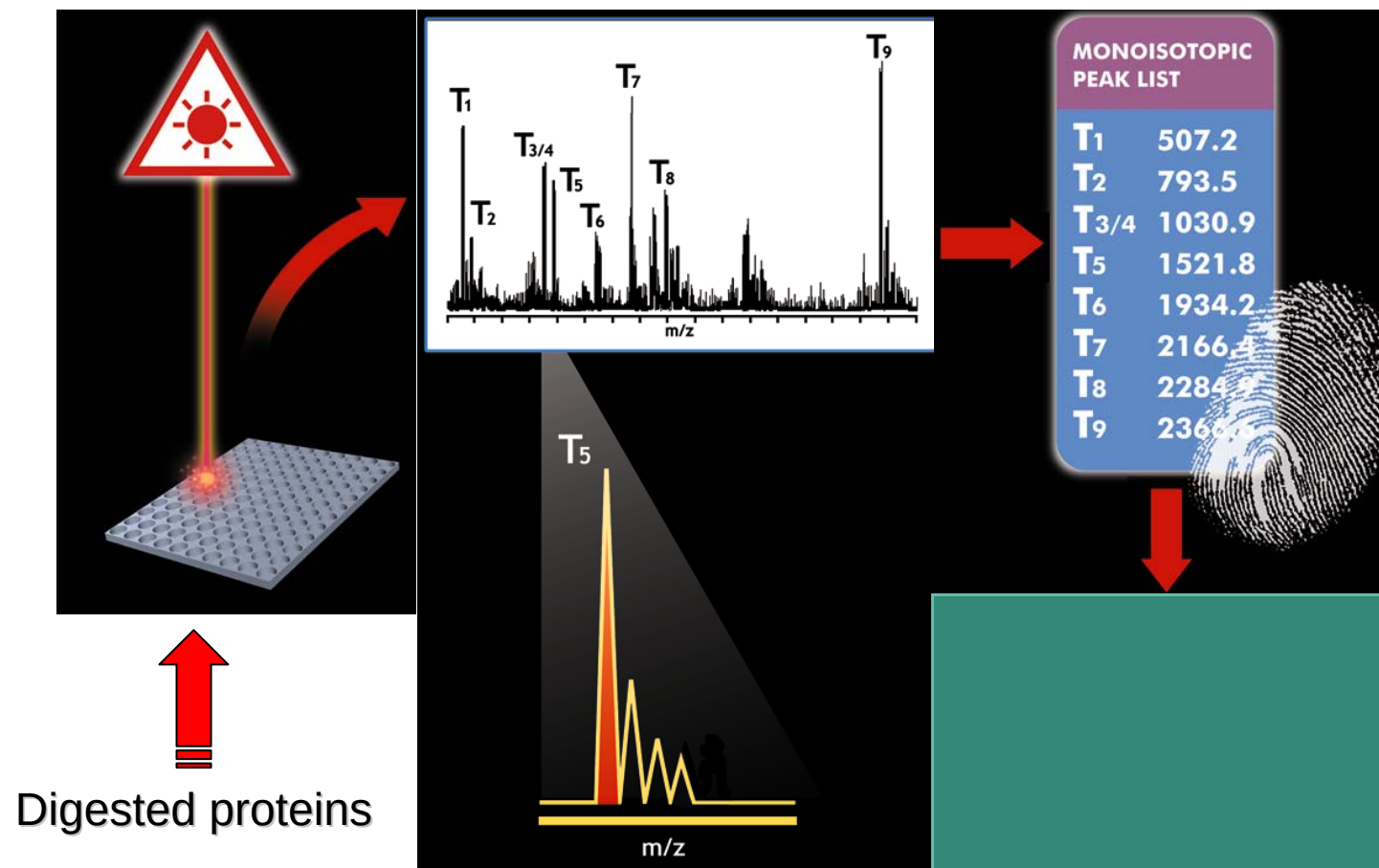
Search Form Defaults: Follow this link to save your preferred search form defaults as a browser cookie.



Peptide mapping分析for Protein ID



■ Peptide mass fingerprinting

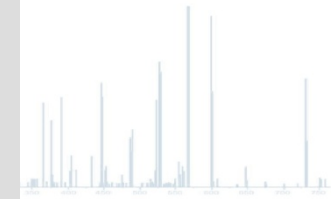


Peptide Mass Fingerprint



Your name	jimmy		Email	jimmy.tsai@mass-solutions.com	
Search title					
Database(s)	SwissProt NCBIInr contaminants cRAP MSDB		Enzyme	Trypsin ▾	
			Allow up to	1 ▾ missed cleavages	
Taxonomy	All entries ▾				
Fixed modifications	--- none selected --- ☐ Display all modifications		> <	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term) Ammonia-loss (N-term C) Biotin (K) Biotin (N-term) Carbamyl (K) Carbamyl (N-term) Carboxymethyl (C)	
Variable modifications	Carbamidomethyl (C) Deamidated (NQ) Oxidation (M)		> <		
Protein mass	kDa		Peptide tol. ±	0.3 Da ▾	
Mass values	☒ MH ⁺ ☐ M _r ☐ M-H ⁻		Monoisotopic	☒ Average ☐	
Data file	Browse...				
Query NB Contents of this field are ignored if a data file is specified.					
Decoy	☐		Report top	AUTO ▾ hits	
Start Search ...			Reset Form		

Peptide Mass Fingerprint

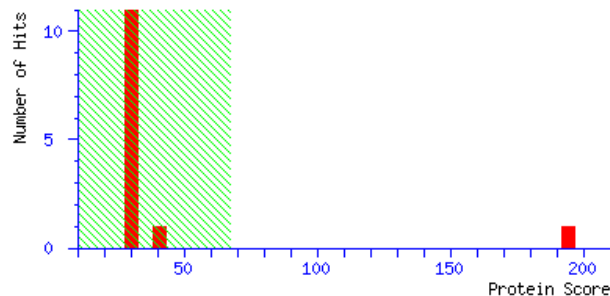


MASCOT Mascot Search Results

User :
Email :
Search title : Peptide Mass Fingerprint Example
Database : SwissProt 51.6 (257964 sequences; 93
Timestamp : 19 Feb 2007 at 14:08:10 GMT
Top Score : 194 for **PML_HUMAN**, Probable transcri

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, where P is the probability that the observed protein score is due to chance.
Protein scores greater than 67 are significant ($p < 0.05$).



Mascot Search Results: Protein View

401	KASFEAASIP	RDFIDVDLPE	EAERVKAQVQ	ALGLAEQPM	AVVQSVPGAH
451	PVPVYAFSIK	GPSYGEDVSN	TTTAQKRKCS	QTQCPRKVIK	MESEEGKEAR
501	LARSSPEQPR	PSTSKAVSPP	HLDGPPSPRS	PVIGSEVFLP	NSNHVASGAG
551	EAERVVVVIS	SSESDAENS	SSRELDSSS	ESSDLQLEGP	STLRVLDENL
601	ADPQAEDRPL	VFFDLKIDNE	TQKISQLAAV	NRESKFRVVI	QPEAFFSIYS
651	KAVSLEVGLQ	HFLSFLSSMR	RPILACYKLW	GPGLPNFFRA	LEDINRLWEF
701	QEAI SGFLAA	LPLIRERVPG	ASSFKLKNLA	QTYLARNMSE	RSAMAAVLAM
751	RDLCLLEVS	PGPQLAQHVY	PFSSQLQCFAS	LQPLVQAQAVL	PRAEARLLAL
801	HNVSMELLS	AHRRDRQGGL	KKYSRYLSLQ	TTTLPPAQPA	FNLQALGTYY
851	EGLLEGPALA	RAEGVSTPLA	GRGLAERASQ	QS	

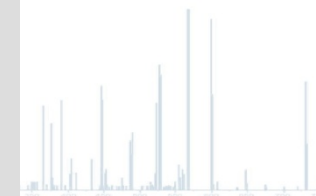
Show predicted peptides also

Sort Peptides By: ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
8 - 33	2882.5000	2881.4927	2881.3777	0.1150	0	R.SPRPQQDPAEPQETMPPE
34 - 44	1182.4400	1181.4327	1181.5677	-0.1349	0	R.QPSPSPSPPTER.A
45 - 56	1423.5200	1422.5127	1422.6779	-0.1652	0	R.APASEEEFQFLR.C
161 - 170	1191.5000	1190.4927	1190.6520	-0.1592	0	K.HEARPLAELR.N
308 - 315	1000.3300	999.3227	999.3967	-0.0740	0	R.DYEEMASR.L
319 - 325	814.4300	813.4227	813.4708	-0.0481	0	R.LDAVLQR.I

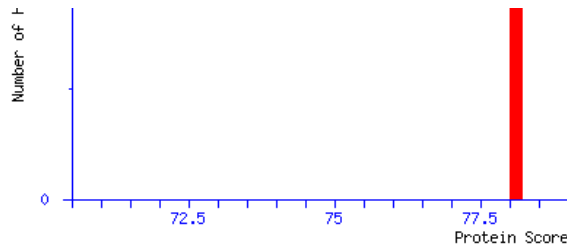
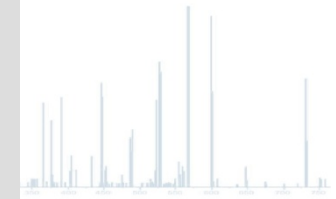
No MS/MS information

Sequence Query



Your name	jimmy	Email	jimmy.tsai@mass-solutions.com
Search title			
Database(s)	Human_EST Fungi_EST Environmental_EST SwissProt NCBIInr	Enzyme	Trypsin
		Allow up to	1 missed cleavages
		Quantitation	None
Taxonomy	All entries		
Fixed modifications	--- none selected ---	> <	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term) Ammonia-loss (N-term C) Biotin (K) Biotin (N-term) Carbamyl (K) Carbamyl (N-term) Carboxymethyl (C)
	Display all modifications ☐		
Variable modifications	Carbamidomethyl (C) Deamidated (NQ) Oxidation (M)	> <	
Peptide tol. ±	0.3 Da # ¹³ C 0	MS/MS tol. ±	0.3 Da
Peptide charge	1+, 2+ and 3+	Monoisotopic	☒ Average ☐
Query	1454.4 ions(b-610,707,804,1086) ions(y-2909) ions(2106,2632,2545)		
Instrument	ESI-QUAD-TOF		
Decoy	☐	Report top	AUTO hits
Start Search ...		Reset Form	

Sequence Query



Peptide Summary Report

Format As: Peptide Summary

Significance threshold $p < 0.05$ Max. number

Standard scoring ☒ MudPIT scoring ☐ Ions score or

Show pop-ups ☒ Suppress pop-ups ☐ Sort unassigned

Select All Select None Search Selected Archive

1. [CASB_BOVIN](#) Mass: 25091 Score: 78 Matches
Beta-casein precursor - Bos taurus (Bovine)

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta M
☒ 1	1031.4000	2060.7854	1980.8548	79.9306

Mascot Search Results: Peptide View - Windows Internet Explorer

http://60.250.102.233/mascot/cgi/peptide_view.i

我的最愛 建議的網站 自訂連結 取得更多附加元件

Mascot Search Results: Peptide View

Peptide view

MS/MS Fragmentation of **FQSEEQQTDELQDK**
Found in **CASB_BOVIN**, Beta-casein precursor - Bos taurus (Bovine)

Match to Query 1: 2060.785448 from(1031.400000,2+) etag(977.4,[Q|K][Q|K]EE,1619.7)

Monoisotopic mass of neutral peptide Mr(calc): 1980.8548
Unsuspected modification: 79.9306 Da, located in the region N-term to S3
Ions Score: 78 Expect: 1.7e-006 ([help](#))

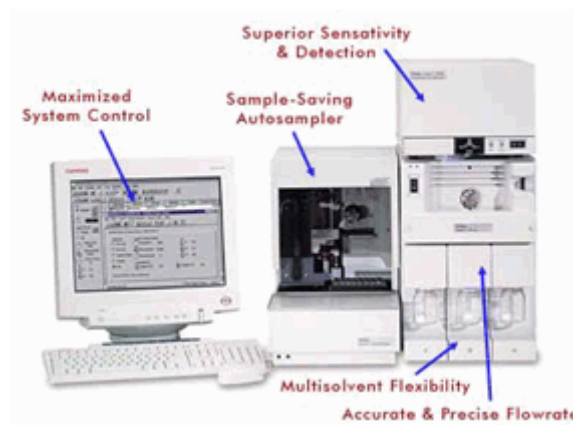
#	b	b ⁺⁺	b [*]	b ⁺⁺	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ⁺⁺
1	148.0757	74.5415					F				
2	276.1343	138.5708	259.1077	130.0575			Q	1834.7937	917.9005	1817.7672	909.3872
3	363.1663	182.0868	346.1397	173.5735	345.1557	173.0815	S	1706.7351	853.8712	1689.7086	845.3579
4	492.2089	246.6081	475.1823	238.0948	474.1983	237.6028	E	1619.7031	810.3552	1602.6766	801.8419
5	621.2515	311.1204	604.2240	302.6161	603.2400	302.1241	E	1400.6605	745.8230	1473.6340	737.2206

完成 網際網路 | 受保護模式: 啟動 100%

LC/ESI/MS/MS for Protein ID



Digested proteins



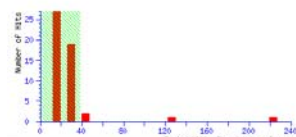
Waters
CapLC pump

MASCOT Search Results

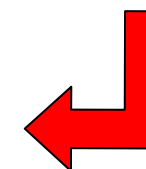
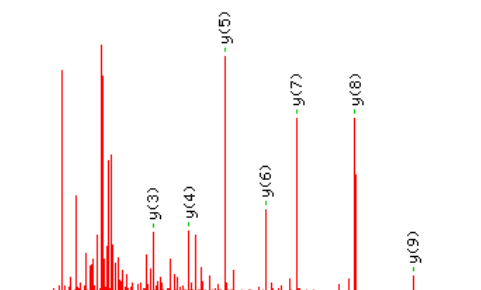
User : JDDMY
Email : jingtao@sinica.edu.tw
Search title :
MS data file : C:\Documents and Settings\jimmy.MICROMASS\My Documents\1119-V14.phl
Database : NCBI nr 20041117 (2171938 sequences; 736969646 residues)
Taxonomy : Viridiplantae (Green Plants) (185653 sequences)
Timestamp : 22 Nov 2004 at 02:28:26 GMT
Significant hits: [gi121263](#) 23 kDa OEC protein [Spinacia oleracea]
[gi182498](#) photosystem II oxygen-evolving complex protein 2 - rice (fragment)
[gi17843103](#) hypothetical protein [Arabidopsis thaliana]

Probability Based Mascot Score

Ions score is $-10 \times \log(P)$, where P is the probability that the observed match is a random event.
Individual ions scores > 38 indicate identity or extensive homology ($p < 0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



MASCOT results



MS/MS data processing and
database searching

LC/MS/MS Peak List 範例 .pkl format



Fragment ion m/z

940719NTU16 7 HVR.pkl - 記事本

檔案(F) 編輯(E) 格式(O) 檢視(V) 說明(H)

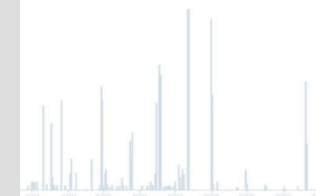
756.0574	363.0883	1
63.7221	1.1027	
91.0484	2.1737	
97.0503	1.1027	
120.4775	1.1027	
129.0959	10.8066	
130.0897	2.1027	
132.0688	3.1434	
133.8813	1.1027	
140.9918	1.1027	
145.0782	1.1027	
153.0452	2.1364	
155.0382	3.1828	
157.1126	1.1027	
159.0871	8.6121	
159.2573	0.6716	
160.1018	2.2054	
167.0827	1.1027	
168.0473	1.1027	
170.0706	1.0795	
170.2327	1.0795	
170.5988	1.1027	
171.0917	5.3412	
172.0998	1.1027	
175.0002	1.1027	
178.0165	1.1027	
180.0442	2.1020	
180.6577	1.1027	

charge

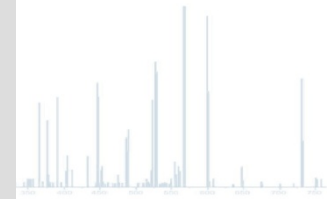
intensity

m/z

LC/MS/MS Peak List 範例 .mgf format



MS/MS Ions Search



MASCOT MS/MS Ions Search

Your name	jimmy	Email	jimmy.tsai@mass-solutions.com
Search title			
Database(s)	Human_EST Fungi_EST Environmental_EST SwissProt NCBIInr	Enzyme	Trypsin
		Allow up to	1 missed cleavages
		Quantitation	None
Taxonomy	All entries		
Fixed modifications	--- none selected ---	> <	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term) Ammonia-loss (N-term C) Biotin (K) Biotin (N-term) Carbamyl (K) Carbamyl (N-term) Carboxymethyl (C)
	Display all modifications ☐		
Variable modifications	Carbamidomethyl (C) Deamidated (NQ) Oxidation (M)	> <	
Peptide tol. ±	0.3 Da	# ¹³C	0
		MS/MS tol. ±	0.3 Da
Peptide charge	1+, 2+ and 3+		
		Monoisotopic	☒ Average ☐
Data file	瀏覽...		
Data format	Micromass (.PKL)	Precursor	m/z
Instrument	ESI-QUAD-TOF	Error tolerant	☐
Decoy	☐	Report top	AUTO hits
	Start Search ...		Reset Form

- | Search Parameters
- | database
- | taxonomy
- | enzyme
- | missed cleavages
- | fixed modifications
- | variable modifications
- | protein MW
- | protein pl
- | estimated mass measurement error

Database search



{MATRIX} Mascot Search Results

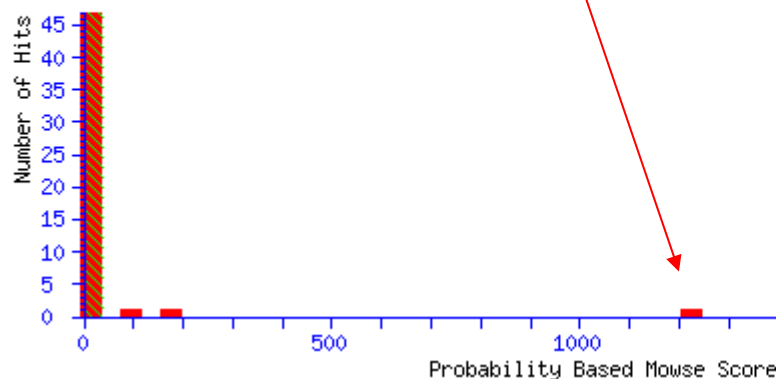
User :
Email :
Search title : MS/MS Example
MS data file : C:\Auto MSMS output\Sample 1.pk1
Database : SwissProt 51.6 (257964 sequences; 93947433 residues)
Timestamp : 19 Feb 2007 at 14:09:47 GMT
Protein hits : CH60_HUMAN 60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa c
CH60_DROME 60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa c
CH60_CAEEL Chaperonin homolog Hsp-60, mitochondrial precursor (Heat shock prote
CH60_XANAC 60 kDa chaperonin (Protein Cpn60) (groEL protein) - Xanthomonas axor

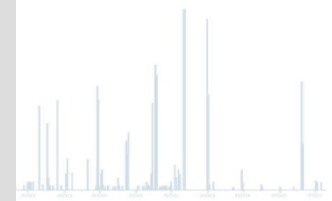
Probability Based Mowse Score

Ions score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event.

Individual ions scores > 38 indicate identity or extensive homology ($p < 0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



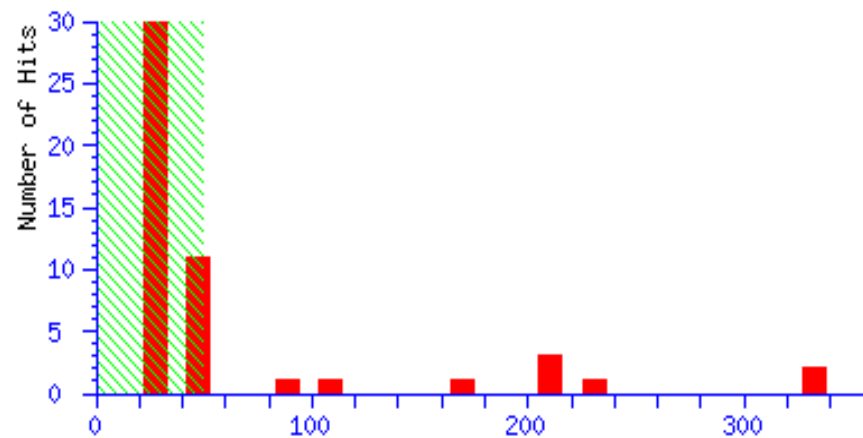


Probability Based Mowse Score

Ions score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event.

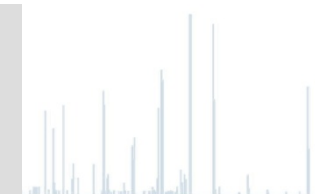
Individual ions scores > 49 indicate identity or extensive homology ($p < 0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



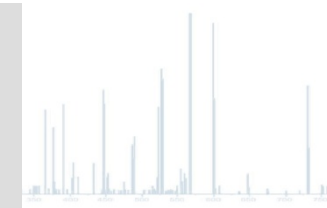
Probability based search algorithm –

Typically 95% confidence level is accepted



1. [CH60_HUMAN](#) Mass: 61016 Score: 1225 Matches: 31(27) Sequences: 19(17)
 60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa chaperonin) (CPN60) (Heat shock
☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
☒ 11	417.1822	832.3498	832.3828	-0.0329	0	45	0.016	1		K.APGFGDNR.K
☒ 12	422.7433	843.4720	843.5066	-0.0346	0	46	0.017	1	U	K.VGEVIVTK.D
☒ 13	430.7328	859.4510	859.4837	-0.0327	0	36	0.15	1	U	K.IPAMTIAK.N + Oxidation (M)
☒ 15	451.2499	900.4853	900.5280	-0.0428	0	52	0.0039	1	U	K.LSDGVAVLK.V
☒ 16	456.7806	911.5467	911.5804	-0.0337	0	59	0.00056	1	U	K.VGLQVAVK.A
☒ 21	480.7447	959.4748	959.5036	-0.0288	0	45	0.017	1	U	R.VTDALNATR.A
☒ 24	595.7855	1189.5565	1189.6012	-0.0447	0	(57)	0.0011	1	U	K.EIGNIISDAMK.K
☒ 25	603.7720	1205.5294	1205.5962	-0.0668	0	60	0.00048	1	U	K.EIGNIISDAMK.K + Oxidation
☒ 26	608.3099	1214.6052	1214.6507	-0.0455	0	73	2.2e-05	1	U	K.NAGVEGSLIVEK.I
☒ 27	617.2857	1232.5569	1232.5885	-0.0316	0	81	4e-06	1	U	K.VGGTSDVEVNEK.K
☒ 31	672.8375	1343.6605	1343.7085	-0.0480	0	64	0.00016	1	U	R.TVIIEQSWGSPK.V
☒ 34	714.8884	1427.7623	1427.8058	-0.0435	0	(65)	0.00014	1	U	R.GVMLAVDAVIAELK.K
☒ 35	714.8938	1427.7730	1427.8058	-0.0327	0	(73)	2.1e-05	1	U	R.GVMLAVDAVIAELK.K
☒ 36	722.8849	1443.7552	1443.8007	-0.0455	0	75	1.2e-05	1	U	R.GVMLAVDAVIAELK.K + Oxidati
☒ 37	722.8934	1443.7722	1443.8007	-0.0285	0	(73)	2.2e-05	1	U	R.GVMLAVDAVIAELK.K + Oxidati
☒ 39	752.8643	1503.7141	1503.7490	-0.0349	0	90	4.3e-07	1	U	K.TLNDELEIIEGMK.F
☒ 40	760.8461	1519.6777	1519.7439	-0.0662	0	(89)	4.7e-07	1	U	K.TLNDELEIIEGMK.F + Oxidatic
☒ 45	640.3281	1917.9625	1918.0636	-0.1010	0	102	2.1e-08	1	U	K.ISSIQSIVPALEIANHR.K
☒ 46	960.0327	1918.0509	1918.0636	-0.0127	0	(87)	5.1e-07	1	U	K.ISSIQSIVPALEIANHR.K
☒ 48	1019.5106	2037.0067	2037.0153	-0.0087	0	52	0.0015	1	U	R.IQEIIQLDVTTSEYEK.E
☒ 51	1057.0537	2112.0929	2112.1323	-0.0394	0	116	6.8e-10	1	U	R.ALMLQGVDLLADAVAVTMGPK.G
☒ 52	1065.0399	2128.0653	2128.1272	-0.0619	0	(72)	1.7e-05	1	U	R.ALMLQGVDLLADAVAVTMGPK.G +
☒ 53	1065.0623	2128.1100	2128.1272	-0.0172	0	(26)	0.63	1	U	R.ALMLQGVDLLADAVAVTMGPK.G +
☒ 54	1073.0477	2144.0809	2144.1221	-0.0412	0	(93)	1.3e-07	1	U	R.ALMLQGVDLLADAVAVTMGPK.G +



Protein View

1. **CH60_HUMAN** Mass: 61 60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa cl
60 kDa heat shock prot Found in search of C:\Auto MSMS output\Sample 1.pkl

☐ Check to include this Nominal mass (M_r): 61016; Calculated pI value: 5.70

NCBI BLAST search of **CH60_HUMAN** against nr

Unformatted [sequence string](#) for pasting into other applications

Query	Observed	Mr (kDa)	
☒ 11	417.1822	832.	Taxonomy: Homo sapiens
☒ 12	422.7433	843.	
☒ 13	430.7328	859.	Variable modifications: Oxidation (M)
☒ 15	451.2499	900.	Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
☒ 16	456.7806	911.	Sequence Coverage: 49%

☒ [21](#) 480.7447 959. Matched peptides shown in **Bold Red**

☒ 24	595.7855	1189.	1 MLRLPTVFRQ MRPVSRVLAP HLTRAYAKDV KFGADAR ALM LQGVDLLADA
☒ 25	603.7720	1205.	51 VAVTMGPKGR TVIIEQSWGS PKVTKDGVT AKSIDLKDKY KNIGAKLVQD
☒ 26	608.3099	1214.	101 VANNTNEEAG DGTTTATVLA RSIAKEGF ISKGANPVEI RRGVMLAVDA
☒ 27	617.2857	1232.	151 VIAELKKQSK PVTTP EEIAQ VATISANGDK EIGNIISDAM KKVGRKGVIT
☒ 31	672.8375	1343.	201 VKDGK TLNDE LEIIEGMKFD RGYISPYFIN TSKGQKCEFQ DAYVLLSEKK
☒ 34	714.8884	1427.	251 ISSIQSIVPA LEIAN AHRKP LVIIAEDVDG EALSTLVLNR LKVGLQVVAV
☒ 34	714.8884	1427.	301 KAPGFGDNRK NQLKD MAIAT GGAVFGEEGL TLNLEDVQPH DLGKVG EVIV
☒ 34	714.8884	1427.	351 TKDDAMLLKG KGDKAQIEKR IQEII EQLDV TTSEYEKEKL NERLAKLSDG
☒ 34	714.8884	1427.	401 VAVLKVG GTS DVEVNEK KDR VTDALN ATRA AVEEGIVLGG GCALLRCIPA
☒ 34	714.8884	1427.	451 LD SLTPANED QKIGIEIIKR TLKIPAM TIA KNAGVEG SLI VEKIMQ SSSE
☒ 34	714.8884	1427.	501 VG YDAMAGDF VNMVEKGIID PTKVVRTALL DAAGV ASLLT TA EVVVTEIP
☒ 34	714.8884	1427.	551 K EEKDPGMGA MGGMGGGMGG GMF



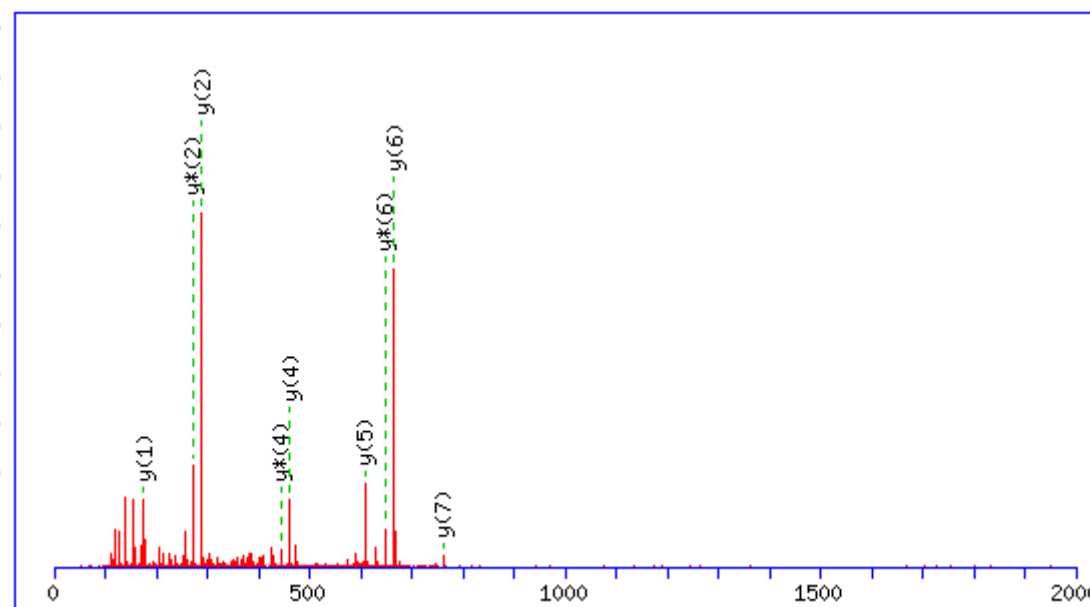
Peptide View

MS/MS Fragmentation of **APGFGDNR**

1. [CH60_HUMAN](#) Mass: 610 Found in **CH60_HUMAN**, 60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa heat shock protein)

☐ Check to include this Match to Query 11: 832.349828 from(417.182190,2+)
Data file C:\Auto MSMS output\Sample 1.pkl

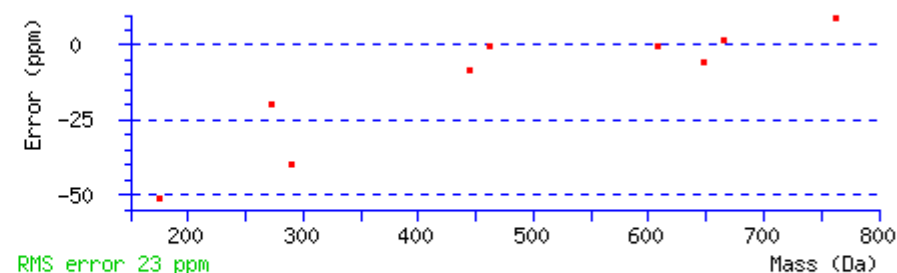
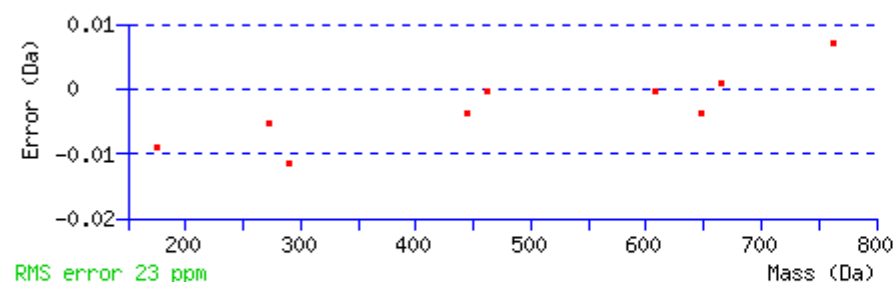
Query	Observed	Mr (e)	Click mouse within plot area to zoom in by factor of two about that point	P
☒ 11	417.1822	832.	Or, Plot from 0 to 2000 Da Full range	K
☒ 12	422.7433	843.	Label all possible matches ☐ Label matches used for scoring ☒	K
☒ 13	430.7328	859.		K
☒ 15	451.2499	900.		K
☒ 16	456.7806	911.		K
☒ 21	480.7447	959.		R
☒ 24	595.7855	1189.		K
☒ 25	603.7720	1205.		K
☒ 26	608.3099	1214.		K
☒ 27	617.2857	1232.		K
☒ 31	672.8375	1343.		R
☒ 34	714.8884	1427.		R
☐ --	---	---		-



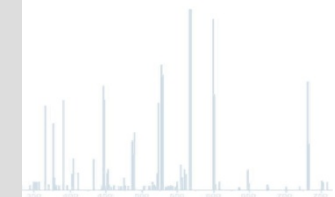


Monoisotopic mass of neutral peptide Mr(calc): 832.3828
 Ions Score: 45 Expect: 0.016
 Matches : 9/58 fragment ions using 16 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ⁺⁺⁺	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ⁺⁺⁺	y ⁰	y ⁰⁺⁺	#
1	72.0444	36.5258					A							8
2	169.0972	85.0522					P	762.3529	381.6801	745.3264	373.1668	744.3424	372.6748	7
3	226.1186	113.5629					G	665.3002	333.1537	648.2736	324.6404	647.2896	324.1484	6
4	373.1870	187.0972					F	608.2787	304.6430	591.2522	296.1297	590.2681	295.6377	5
5	430.2085	215.6079					G	461.2103	231.1088	444.1837	222.5955	443.1997	222.1035	4
6	545.2354	273.1214			527.2249	264.1161	D	404.1888	202.5980	387.1623	194.0848	386.1783	193.5928	3
7	659.2784	330.1428	642.2518	321.6295	641.2678	321.1375	N	289.1619	145.0846	272.1353	136.5713			2
8							R	175.1190	88.0631	158.0924	79.5498			1



NCBI **BLAST** search of [APGFGDNR](#)
 (Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
 Other BLAST [web gateways](#)



2. [gi|13591902](#) Mass: 103476 Score: 136 Queries matched: 4
actinin, alpha 1 [Rattus norvegicus]

☐ Check to include this hit in error tolerant search

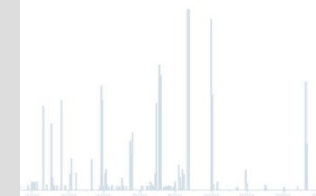
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒ 22	588.3079	1174.6012	1174.5804	0.0208	0	28	0.11	1	K.EGLLLWCQR.K
☒ 49	693.8995	1385.7844	1385.7667	0.0178	0	57	0.00011	1	R.VGWEQLLTIIAR.T
☒ 196	1170.0564	2338.0982	2338.1804	-0.0822	0	51	0.00019	1	K.IDQLEGDHQLIQEALIFDNK.H
☒ 197	780.3976	2338.1710	2338.1804	-0.0095	0	(29)	0.027	1	K.IDQLEGDHQLIQEALIFDNK.H

Proteins matching the same search
[gi|38018016](#) Mass: 10604
brain-specific alpha actinin
[gi|49256643](#) Mass: 10312
Actn1 protein [Rattus norvegicus]

Top scoring peptide matches to query 49
Score greater than 28 indicates homology
Score greater than 30 indicates identity
Status bar shows all hits for this peptide

Score	Delta	Hit	Protein	Peptide
56.5	0.02	2	gi 13591902	R.VGWEQLLTIIAR.T
12.0	0.05			K.WTDSALVLSPAAR.N
5.2	0.06			K.TQLQQIAGKDER.V
4.7	-0.02			R.QLNLITMLAIR.V
4.3	0.08			K.ADGSLMDPRVGIR.S
4.3	-0.04			K.RGVLLMLFGGVPK.T
3.4	0.03			R.TGEAIVDAALSALR.Q
2.9	-0.01			K.VEPPPAKVPEVPK.K
0.2	0.05			K.SRVAAQNSAEVVR.C
0.2	0.04			R.VARAMSIPTSPTR.V

Each MSMS (each query number) has an individual corresponding table



3. [gi|223556](#) Mass: 50906 Score: 119 Queries matched: 2
tubulin alpha

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒ 63	729.4404	1456.8662	1456.8613	0.0050	0	56	9.8e-05	1	R.LIGQIVSSITASLR.F
☒ 205	1205.0907	2408.1668	2408.2012	-0.0343	0	63	1.4e-05	1	R.FDGALNVDLTEFQTNLVPYPR.I

7. [gi|17921989](#) Mass: 50646 Score: 102 Queries matched: 2
tubulin, alpha 1 [Homo sapiens]

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒ 91	793.3776	1584.7406	1584.7282	0.0124	0	39	0.0049	1	R.SIQFVDWCPTGFK.V
205	1205.0907	2408.1668	2408.2012	-0.0343	0	63	1.4e-05	1	R.FDGALNVDLTEFQTNLVPYPR.I

Top scoring peptide matches to query 205
Score greater than 27 indicates identity
Status bar shows all hits for this peptide

Score	Delta	Hit	Protein	Peptide
62.8	-0.03	3+	gi 223556	R.FDGALNVDLTEFQTNLVPYPR.I

Bold: the first time a particular match appears in the report

Red: the first ranking peptide match appears



Protein modifications



MASCOT MS/MS Ions Search

Your name	cindy	Email	l3891112@ccmail.ncku.edu.tw
Search title			
Database	NCBIInr		
Taxonomy	 Rattus		
Enzyme	Trypsin	Allow up to	1 missed cleavages
Fixed modifications	Amidated (Protein C-term) Ammonia-loss (N-term C) Biotin (K) Biotin (N-term) Carbamidomethyl (C)		
Variable modifications	Methylthio (C) NIPCAM (C) Oxidation (HW) Oxidation (M) Phospho (ST)		

8. [gi|21707902](#) **Mass:** 71599 **Score:** 83 **Queries matched:** 2
CTTN protein [Homo sapiens]

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒ 133	795.8682	1589.7218	1589.7014	0.0204	0	44	0.02	1	R.LPSSPVYEDAASFK.A + Phospho (ST)
☒ 173	907.9363	1813.8580	1813.8247	0.0333	0	40	0.041	1	K.TQTPVSPAPQPTTEER.L + Phospho (ST)

Export search result



Format As Select Summary (protein hits) ▼ [Help](#)

Peptide Summary
Select Summary (protein hits)
Select Summary (unassigned)
Export Search Results

Max. number of hits

Spring ☒ Ions score or expect cut-off Show sub-sets

Show pop-ups ☒ Suppress pop-ups ☐ Sort unassigned Require bold red ☒

Microsoft Excel - F001754[1]

檔案(F) 編輯(E) 檢視(V) 插入(I) 格式(O) 工具(T) 資料(D) 視窗(W) 說明(H) Adobe PDF(E)

輸入需要解答的問題

新細明體 12 B I U

繁 繁轉簡 簡 簡轉繁 英 英譯繁 繁 繁譯英 英 英譯簡 簡 簡譯英 日 日譯繁 繁 繁譯日 日 日譯簡 簡 簡譯日

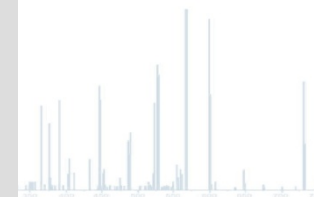
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
46	Protein hits	-----														
47																
48	prot_hit_n	prot_acc	prot_desc	prot_score	prot_mass	prot_match	pep_query	pep_rank	pep_isbold	pep_exp_n	pep_exp_r	pep_exp_z	pep_calc_r	pep_delta	pep_miss	pep_score
49	1	ALBU_BO	Serum albumin precurs	5024	71244	316	2881	2	1	609.317	608.3097	1	608.2806	0.0291	0	23.9
50	1						2883	2	1	609.343	608.3357	1	608.2806	0.0551	0	21.1
51	1						2884	1	1	609.347	608.3397	1	608.2806	0.0591	0	28.7
52	1						2889	1	1	609.551	608.5437	1	608.2806	0.2631	0	20.5
53	1						3195	1	1	660.128	659.1207	1	659.349	-0.2283	0	26.8
54	1						3199	1	1	660.385	659.3777	1	659.349	0.0287	0	29.6
55	1						3200	1	1	660.396	659.3887	1	659.349	0.0397	0	25.3
56	1						3204	1	1	660.492	659.4847	1	659.349	0.1357	0	20.2
57	1						3544	1	1	689.446	688.4387	1	688.3656	0.0731	0	20.7
58	1						3545	1	1	689.468	688.4607	1	688.3656	0.0951	0	20.7
59	1						3648	1	1	706.47	705.4627	1	705.3479	0.1148	0	22.8
60	1						3649	2	1	706.501	705.4937	1	705.3479	0.1458	0	21.7
61	1						3888	1	1	752.391	751.3837	1	751.35	0.0337	0	20.5
62	1						3889	1	1	752.431	751.4237	1	751.35	0.0737	0	20.9
63	1						3954	1	1	758.363	757.3557	1	757.4156	-0.0599	0	39.9
64	1						3956	2	1	758.382	757.3747	1	757.4156	-0.0409	0	31.5
65	1						4166	1	1	789.429	788.4217	1	788.4644	-0.0426	0	31.7
66	1						4168	1	1	789.645	788.6377	1	788.4644	0.1734	0	36.6
67	1						4496	1	1	409.3366	816.6586	2	816.4818	0.1769	1	49.7
68	1						4513	1	1	818.521	817.5137	1	817.4181	0.0956	0	22.3
69	1						4515	1	1	409.8921	817.7696	2	817.4181	0.3515	0	36.8
70	1						4520	1	1	409.9511	817.8876	2	817.4181	0.4695	0	33.2

就緒

開始 志聖工... MST PR... 2 Micro... Windows... 2 Intern... MST - O... Microsoft... Microsoft... CH 下午 05:13

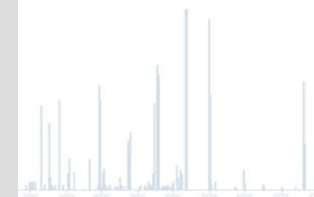
For the publication guideline

What's the difference between public web version and in-house version?



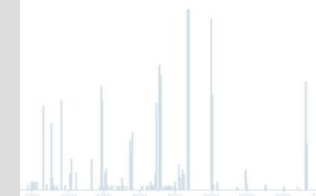
- 1.Query number:
web : 10MB or 1200 spectra in a single MS/MS search
in-house : no limitation
- 2.Searching Databases:
web : adding databases is not available
in-house : users can setup their own databases
- 3.Enzyme :
web : can't perform "no enzyme" search
in-house : no limitation
- 4.modifications :
web : 3 variable modification
in-house : no limitation

What's the difference between public web version and in-house version?



- 5.Quantitation:
 - web : can only use default quantitation methods, and can't co-operate with MASCOT Distiller
 - in-house : no limitation
- 6.Security control :
 - web : N/A
 - in-house : administrator can config all user's rights to use MASCOT in-house server, and search results handling

Mascot進階功能



MASCOT MS/MS Ions Search

Your name	Cindy	Email	shengyu.huang@mass-solutior
Search title			
Database	NCBIInr		
Taxonomy	 Rattus		
Enzyme	Trypsin	Allow up to	1 missed cleavages
Fixed modifications	Amidated (Protein C-term) Ammonia-loss (N-term C) Biotin (K) Biotin (N-term) Carbamidomethyl (C)		
Variable modifications	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term)		
Quantitation	None		
Peptide tol. ±	None		
Peptide charge	iTRAQ 4plex iTRAQ 8plex 18O corrected multiplex SILAC K+6 R+6 multiplex TMT 6plex		
Data file	ICAT ABI Cleavable [MD] ICPL duplex pre-digest [MD] ICPL duplex post-digest [MD] SILAC K+6 R+10 [MD]		
Data format	18O corrected [MD] 15N Metabolic [MD]		
Instrument	Start Search ...		
Decoy	Reset Form		
MS/MS tol. ±	0.2	Da	
Monoisotopic	☒ Average ☐		
Precursor	m/z		
Error tolerant	☐		
Report top	AUTO hits		

Search Result

Peptide Summary Report (Time flies like an arrow) - Microsoft Internet Explorer

檔案(F) 編輯(E) 檢視(V) 我的最愛(A) 工具(T) 說明(H)

← 上一頁 → 搜尋 ★ 我的最愛

網址 http://www.matrixscience.com/cgi/master_results.pl?file=../data/P981131.dat

移至 連結 » Norton Internet Security

Mascot Search Results

User : Mel Anogaster
Email : mel@bioc.cam.ac.uk
Search title : Time flies like an arrow
MS data file : U:\Docs\Quantitation\iTRAQ\cantab\denisef28QSTARcor.mgf
Database : SwissProt 51.6 (257964 sequences; 93947433 residues)
Quantitation : iTRAQ 4plex [method details](#)
Applied Biosystems iTRAQ(TM) reagent
Timestamp : 19 Feb 2007 at 14:31:55 GMT
Protein hits : 115/114 116/114 117/114

0.914	1.438	1.809	APLP_DROME	Apolipoporphins precursor (Retinoid- and fatty acid-binding glycoprotein) [Contains: Apolipoporphin-2
1.761	2.245	3.225	VIT3_DROME	Vitellogenin-3 precursor (Vitellogenin III) (Yolk protein 3) - Drosophila melanogaster (Fruit fly)
1.891	3.221	1.717	PDI_DROME	Protein disulfide-isomerase precursor (EC 5.3.4.1) (PDI) - Drosophila melanogaster (Fruit fly)
1.020	1.191	0.684	UGGG_DROME	UDP-glucose:glycoprotein glucosyltransferase precursor (EC 2.4.1.-) (UDP--Glc:glycoprotein glucosyl
0.846	1.656	3.233	EF11_DROME	Elongation factor 1-alpha (EF-1-alpha) (50 kDa female-specific protein) - Drosophila melanogaster (
1.096	4.004	6.741	TOP2_DROME	DNA topoisomerase 2 (EC 5.99.1.3) (DNA topoisomerase II) - Drosophila melanogaster (Fruit fly)
0.969	1.221	2.165	RL4_DROME	60S ribosomal protein L4 (L1) - Drosophila melanogaster (Fruit fly)
1.042	2.008	3.426	VDAC_DROME	Voltage-dependent anion-selective channel (Porin) (DmVDAC) - Drosophila melanogaster (Fruit fly)
1.422	1.906	1.335	HSP7C_DROME	Heat shock 70 kDa protein cognate 3 precursor (78 kDa glucose-regulated protein homolog) (GRP 78) (
1.090	1.839	1.182	YL_DROME	Putative vitellogenin receptor precursor (Protein yolkless) (YL) - Drosophila melanogaster (Fruit f
1.411	2.166	0.928	SPTCA_DROME	Spectrin alpha chain - Drosophila melanogaster (Fruit fly)
0.357	0.831	1.733	ATPB_DROME	ATP synthase subunit beta, mitochondrial precursor (EC 3.6.3.14) - Drosophila melanogaster (Fruit f
1.051	2.172	2.501	GBLP_DROME	Guanine nucleotide-binding protein subunit beta-like protein (Receptor of activated protein kinase
0.747	1.363	2.370	RS3_DROME	40S ribosomal protein S3 - Drosophila melanogaster (Fruit fly)
1.174	1.160	1.554	VIT1_DROME	Vitellogenin-1 precursor (Vitellogenin I) (Yolk protein 1) - Drosophila melanogaster (Fruit fly)
1.577	3.060	5.007	RL13_DROME	60S ribosomal protein L13 (BBC1 protein homolog) - Drosophila melanogaster (Fruit fly)
1.214	1.097	0.717	CALR_DROME	Calreticulin precursor (CRP55) (Calregulin) (HACBP) - Drosophila melanogaster (Fruit fly)
0.855	1.919	3.548	KPYK_DROME	Pyruvate kinase (EC 2.7.1.40) (PK) - Drosophila melanogaster (Fruit fly)
0.756	0.752	2.068	PABP_DROME	Polyadenylate-binding protein (Poly(A)-binding protein) (PABP) - Drosophila melanogaster (Fruit fly)
1.256	1.958	2.789	RL9_DROME	60S ribosomal protein L9 - Drosophila melanogaster (Fruit fly)
---	---	---	LAMC1_DROME	Laminin subunit gamma-1 precursor (Laminin B2 chain) - Drosophila melanogaster (Fruit fly)
1.492	1.413	1.066	FAS1_DROME	Fasciclin-1 precursor (Fasciclin I) (FAS I) (FCN) - Drosophila melanogaster (Fruit fly)

完成 開始 Windows Live... 2 Internet E... 收件匣 - Out... Mascot Distiller Microsoft Pow... PChome-Skyp... 20070330 ne... 網路網路 上午 10:42

Search Result



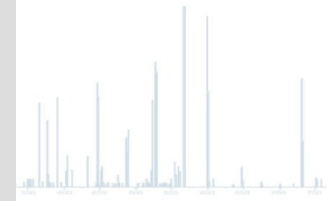
2. [VIT3_DROME](#) Mass: 50436 Score: 515 Queries matched: 19 emPAI: 1.13
Vitellogenin-3 precursor (Vitellogenin III) (Yolk protein 3) - Drosophila melanogaster (Fruit fly)

☐ Check to include this hit in error tolerant search

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.761	10	1.144
	116/114	2.245	10	1.560
	117/114	3.225	11	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	115/114	116/114	117/114	Peptide
☒ 54	534.7767	1067.5388	1067.5491	-0.0103	0	38	0.2	1	1.962	15.529	15.409	K.EQDGF GK.R
☒ 96	625.8919	1249.7692	1249.7638	0.0055	0	36	0.35	1	2.148	2.180	3.490	R.LIQAYVQK.Y
☒ 114	661.8789	1321.7432	1321.7597	-0.0165	0	34	0.52	1	1.929	1.519	2.946	K.YNLQQLQK.N
☒ 118	670.3865	1338.7584	1338.7387	0.0198	0	(19)	17	1	---	---	---	K.LNNYVETAK.A
☒ 119	670.3867	1338.7588	1338.7387	0.0202	0	57	0.0023	1	1.912	1.956	3.475	K.LNNYVETAK.A
☒ 134	705.4144	1408.8142	1408.7805	0.0337	0	68	0.00018	1	1.827	5.873	10.012	R.GDYILEVNAK.S
☒ 143	723.4282	1444.8418	1444.8282	0.0137	0	35	0.4	1	1.174	0.851	1.783	R.NFPAPVANS LK.Q
☒ 147	751.8827	1501.7508	1501.8092	-0.0584	0	18	17	1	---	---	---	K.NAQEQQLK.S
☒ 148	757.9334	1513.8522	1513.8344	0.0179	0	42	0.077	1	3.981	4.020	6.726	R.LENQPLEQ GAK.V
☒ 166	1119.1100	2236.2054	2236.2558	-0.0504	0	61	0.00089	1	1.773	7.411	11.119	K.AASGDLIIIDLGSTLTNFK.R
☒ 172	789.3806	2365.1200	2365.1495	-0.0295	0	70	6.7e-05	1	1.121	0.964	2.058	R.GDAFVDAIHTSTFAMGTPIR.C
☒ 173	789.3896	2365.1470	2365.1495	-0.0025	0	(29)	1.1	1	0.995	1.113	1.314	R.GDAFVDAIHTSTFAMGTPIR.C
☒ 174	789.3903	2365.1491	2365.1495	-0.0004	0	(3)	3.8e+02	1	---	---	---	R.GDAFVDAIHTSTFAMGTPIR.C
☒ 175	789.7163	2366.1271	2365.1495	0.9776	0	(10)	81	1	---	---	---	R.GDAFVDAIHTSTFAMGTPIR.C

何時進行 error tolerance search?



當進行MS/MS Ions search時發現有許多MS/MS spectrum無法比對到任何peptide，而且這些MS/MS spectrum的品質很好時，可能的原因如下：

- 1. 低估誤差值(e.g. 質譜誤差過大時)
- 2. Precursor ion的 charge state判斷錯誤
- 3. 使用的蛋白酶沒有特異性或不佳
- 4. 未知的PTM或化學修飾
- 5. 資料庫中不存在這些peptide序列

MASCOT error tolerance search功能則是針對後三點進行資料庫比對。

PTM: post-translational modification

Error tolerant search



Select All

Select None

Search Selected

☐ Error tolerant

Archive Report

1. [FGF1_HUMAN](#) Mass: 17623 Score: 44 Queries matched: 1 emPAI: 0.22
Heparin-binding growth factor 1 precursor (HBGF-1) (Acidic fibroblast growth factor) (aFGF) (Beta-e

☐ Check to include this hit in error tolerant search or archive report

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒	54	572.3775	1142.7404	1142.5931	0.1473	0	44	0.0015	1	R.ILPDGTVDGTR.D

Peptide matches not assigned to protein hits: (no details means no match)

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒	26	516.2847	1030.5548	1030.5659	-0.0110	0	19	0.54	1	TEDIITAIR
☒	27	516.2908	1030.5670	1030.5659	0.0012	0	17	0.79	1	TEDIITAIR
☐	110	522.5001	1030.5600	1030.5615	0.0015	0	15	0.55	1	TEDIITAIR



{*MATRIX* *SCIENCE*} Mascot Search

Finished uploading search details...

If you license Mascot in-house, you can use Mascot Daemon to automate search submission

Searching...

Search stage: standard search

.....40% complete

.....60% complete

.standard search complete

Search stage: error-tolerant search

.....10% complete

.....30% complete

.....60% complete

.....80% complete



Transgelin (Smooth muscle protein 22-alpha) (SM22-alpha) - Rattus norvegicus (Rat)

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒ 16	602.8030	1203.5914	1203.5958	-0.0043	0	27		1	K_QMEQVAQFLK.A + [-17.0265 at N-term Q]
☒ 17	603.8219	1205.6292	1205.6690	-0.0397	0	91		1	R.TVMALGSLAVTK.N + [+15.9949 at M3]
☒ 18	603.8374	1205.6602	1205.6690	-0.0087	0	(45)		1	R.TVMALGSLAVTK.N + [+15.9949 at M3]
☒ 28	641.2947	1280.5748	1280.5885	-0.0136	0	37	0.0017	1	R.EFTDSQLQEGK.H

20. [ACTA_RAT](#) Mass: 42381 Score: 37 Queries matched: 3 emPAI: 0.10

Actin, aortic smooth muscle (Alpha-actin-2) - Rattus norvegicus (Rat)

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒ 13	581.3078	1160.6010	1160.6111	-0.0100	0	(37)	0.0024	1	K.EITALAPSTMK.I
☒ 15	589.3031	1176.5916	1176.6060	-0.0144	0	42		1	K.EITALAPSTMK.I + [+15.9949 at M10]
☒ 120	1122.9940	2243.9734	2244.0924	-0.1190	0	40		1	K.DLYANNVLSGGTMYPGIADP.M + [+17.0345 at D201]

Possible Assignments:

Oxidation (M) [+15.9949]

Proteins matching the same set of peptides:

[ACTC_RAT](#) Mass: 42334 Score: 37 Queries matched: 3

Actin, alpha cardiac muscle 1 (Alpha-cardiac actin) - Rattus norvegicus (Rat)

[ACTH_RAT](#) Mass: 42249 Score: 37 Queries matched: 3

Actin, gamma-enteric smooth muscle (Smooth muscle gamma actin) (Gamma-2-actin) (Alpha-actin-3) - Rattus norvegicus (Rat)



- $$\frac{\text{no. of id. In decoy database search}}{\text{no. of id. In normal database search}}$$

Automated decoy database search



[K2C4_BOVIN](#) Keratin, type II cytoskeletal 59 kDa, component IV (Fragment) OS=Bos taurus PE=2 SV=1
[K2C72_HUMAN](#) Keratin, type II cytoskeletal 72 OS=Homo sapiens GN=KRT72 PE=1 SV=2
[CJ047_HUMAN](#) Uncharacterized protein C10orf47 OS=Homo sapiens GN=C10orf47 PE=1 SV=2

	SwissProt	Decoy	False discovery rate
Peptide matches above identity threshold	51	0	0.00 %
Peptide matches above homology or identity threshold	74	3	4.05 %

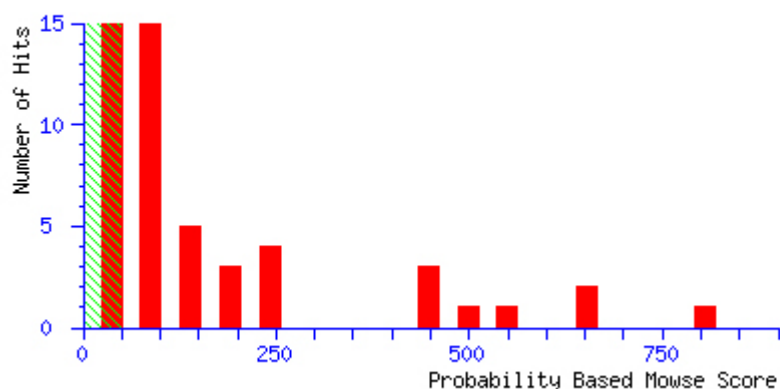
點選後可以看decoy database比對的結果

Probability Based Mowse Score

Ions score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event.

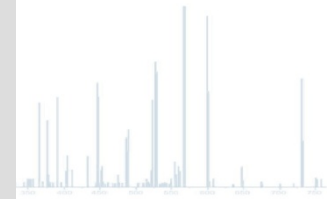
Individual ions scores > 48 indicate identity or extensive homology ($p < 0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



以這張結果為例，利用decoy database進行比對分數達homology的false-positive rate為4.05%，比對分數達identity的false-positive rate為0%

New features in Mascot



- **Protein Family Report**
- **Percolator Support**
- **Search Multiple Databases**
- **Export search results as mzIdentML**
- **Batch automate quantitation with Mascot Daemon**
- **Support for mzML format peak lists**
- **64-bit executables for Windows**
- **Result report caching makes all large reports faster to load**
- **Support for Perl 5.10**
- **Multiplex quantitation now supports isobaric precursors**
- **Reports now show counts of distinct sequences as well as counts of matched spectra**



... and lots more



New reports – load much more rapidly



Caching 20090101\F001451.dat

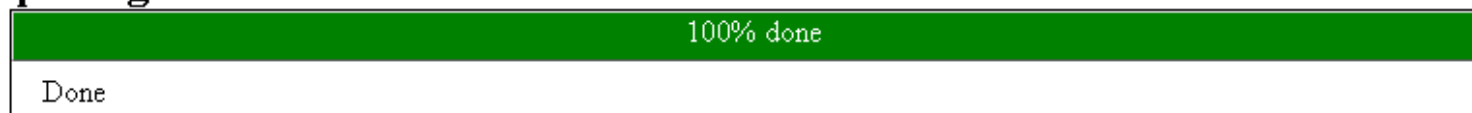
Please wait while the file is being processed. Please *do not*

- close the browser,
- refresh this page, or
- navigate away from this page;

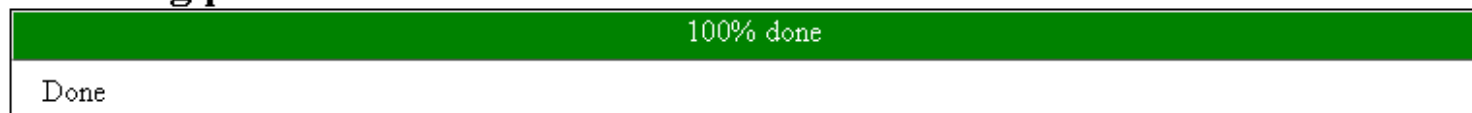
otherwise the process will have to be restarted.

Depending on file size, available memory, CPU and disk performance, and many other factors, indexing may take several minutes, and up to an hour.

Opening file:



Processing protein hits:

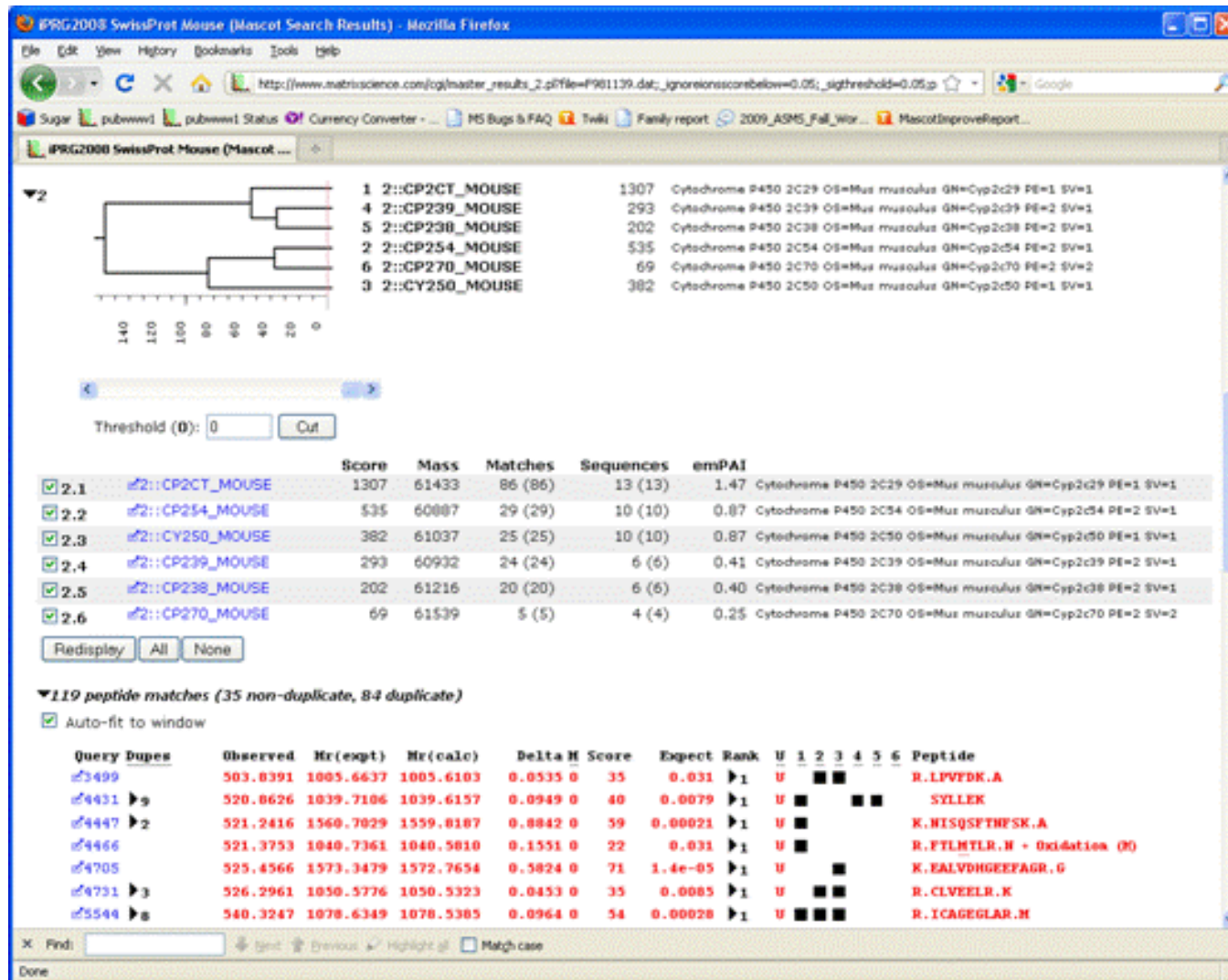


Saving protein families:



Protein Family Report

Protein Family Report



Protein Family Report



MASCOT Search Results

User :
E-mail :
Search title : iPRG2008 SwissProt Mouse
MS data file : D:\iPRG2008\mgf\merged.mgf
Databases : 1: cRAP 20090731 (111 sequences; 37,180 residues)
2: SwissProt 57.14 (514,789 sequences; 181,163,771 residues)
Taxonomy : 1: (none)
2: Mus. (16,273 sequences)
Timestamp : 5 Mar 2010 at 14:04:48 GMT
Warning : No taxonomy indexes for cRAP, taxonomy 'Mus.' ignored. Searching all entries in cRAP

☒ All ☐ Non-significant ☐ Unassigned [\[help\]](#) As

Not what you expected? Try [the select summary](#).

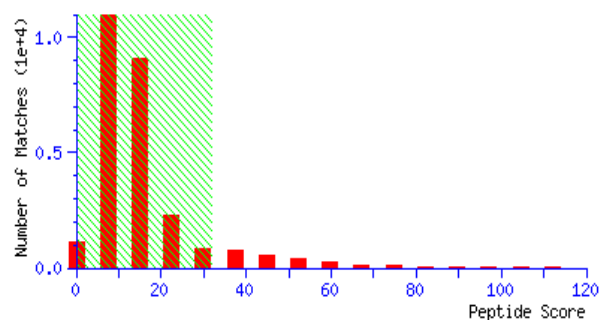
Search parameters

Type of search : MS/MS Ion Search
Enzyme : Trypsin/P
Fixed modifications : [iTRAQ4plex \(K\)](#), [iTRAQ4plex \(N-term\)](#), [Methylthio \(C\)](#)
Variable modifications : [Acetyl \(Protein N-term\)](#), [Gln->pyro-Glu \(N-term Q\)](#), [Oxidation \(M\)](#)
Mass values : Monoisotopic
Protein mass : Unrestricted
Peptide mass tolerance : ± 0.9 Da
Fragment mass tolerance : ± 0.6 Da
Max missed cleavages : 1
Instrument type : ESI-TRAP
Number of queries : 33,191

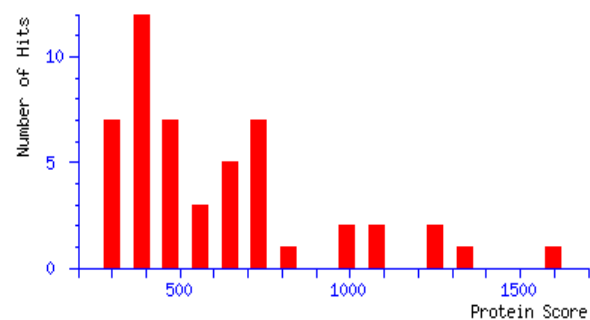
Protein Family Report



Score distribution



Peptide score distribution. Ions score is $-10\log(P)$, where P is the probability that the observed match is a random event. Individual ions scores > 32 indicate **identity** or **extensive homology** ($p < 0.05$).



[Deprecated] Score distribution for family members in the first 50 proteins. Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein families.

Legend

Dupes	Expect	Rank	U	1	2	Peptide	
	0.037	▶	2			GAYSLSLR	significant
	9	▶	1			GFFLFVEGGR	top ranking
	6.4e-05	▶	1			GSSIFGLAPGK	significant and top ranking
	1.3e-06	▶	1	■		SSGTSYPDVLK	peptide is found in all proteins in family member 1
	6.2e-07	▶	1	■		VCNYVSWIK	peptide is found in some but not all proteins in family member 2
	6.4e-05	▶	1	U		GSSIFGLAPGK	unique
▶	2	5.7e-05	▶	1		LNTLETEEWFFK	peptide has two duplicates
	0.18	▶	1			LNTLETEEWFFK	duplicate peptide

Right-facing triangle (▶) in the Dupes or Rank column indicates content that can be expanded by clicking on it. Down-facing triangle (▼) indicates the content is expanded and can be collapsed. For more details about particular columns, see [results format help](#).

Protein Family Report



Protein Family Summary

Significance threshold p< Max. number of families [\[help\]](#)
Ions score or expect cut-off

Proteins (18)

[Quantitation \(20\)](#)

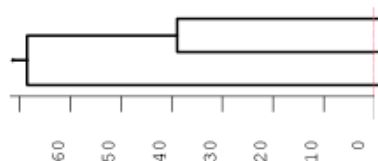
[Unassigned \(3004\)](#)

Protein families 1-10 (out of 18)

10 per page 1 [2](#) [Next](#)

Accession ☐ Match case

►1



1 gi|94733733
3 gi|166795887
2 gi|156713467

568 novel protein similar to vitellogenin 1 (vg1) [Danio rerio]
310 vitellogenin 1 [Danio rerio]
343 vitellogenin 7 [Danio rerio]

►2

gi|226529363

237 dickkopf-related protein 3 [Danio rerio]

►3

gi|41152464

129 ribosomal protein S14 [Danio rerio]

►4

gi|54400698

96 hydroxysteroid (17-beta) dehydrogenase 10 [Danio rerio]

►5

gi|37595356

91 ribosomal protein S3 [Danio rerio]

►6

gi|182889552

88 Bactin1 protein [Danio rerio]

►7

gi|18859307

79 ras-related nuclear protein [Danio rerio]

►8

gi|94536645

54 hypothetical protein LOC678611 [Danio rerio]

►9

gi|47550922

50 heat shock protein 4-like [Danio rerio]

Protein Family Report (Quantitation)



[Proteins \(18\)](#)
[Quantitation \(20\)](#)
[Unassigned \(3004\)](#)

Quantitation overview (20 proteins)

		Score	Mass	Matches	Sequences	emPAI
1.1	gi 94733733	568	149143	26 (18)	14 (11)	0.54 novel protein similar to vitellogenin 1 (vg1) [Danio rerio]
1.2	gi 156713467	343	148739	14 (11)	10 (8)	0.37 vitellogenin 7 [Danio rerio]
1.3	gi 166795887	310	149453	21 (12)	14 (9)	0.36 vitellogenin 1 [Danio rerio]
2	gi 226529363	237	32678	14 (11)	3 (2)	1.31 dickkopf-related protein 3 [Danio rerio]
3	gi 41152464	129	16235	4 (3)	2 (1)	0.32 ribosomal protein S14 [Danio rerio]
4	gi 54400698	96	27040	2 (2)	2 (2)	0.40 hydroxysteroid (17-beta) dehydrogenase 10 [Danio rerio]
5	gi 37595356	91	26862	2 (2)	2 (2)	0.40 ribosomal protein S3 [Danio rerio]
6	gi 182889552	88	41683	7 (6)	4 (3)	0.55 Bactin1 protein [Danio rerio]
7	gi 18859307	79	24445	3 (2)	3 (2)	0.45 ras-related nuclear protein [Danio rerio]
8	gi 94536645	54	50900	7 (2)	6 (2)	0.20 hypothetical protein LOC678611 [Danio rerio]
9	gi 47550833	50	93285	3 (2)	2 (1)	0.05 heat shock protein 4, like [Danio rerio]
10	gi 4504301	48	11360	2 (2)	1 (1)	0.48 histone H4 [Homo sapiens]
11	gi 47086117	45	30271	1 (1)	1 (1)	0.16 ribosomal protein S2 [Danio rerio]
12	gi 41055255	41	8897	2 (1)	2 (1)	0.63 ribosomal protein S21 [Danio rerio]
13	gi 113676895	33	89789	2 (1)	2 (1)	0.05 component of oligomeric golgi complex 5 [Danio rerio]
14	gi 189515664	29	44583	5 (1)	1 (1)	0.11 PREDICTED: similar to neuromedin U receptor 1 [Danio rerio]
15	gi 189523699	27	3645668	26 (1)	20 (1)	PREDICTED: titin a [Danio rerio]
16	gi 189520106	26	82738	1 (1)	1 (1)	0.06 PREDICTED: similar to COBL-like 1 [Danio rerio]
17	gi 41387210	21	22633	2 (1)	2 (1)	0.22 phosphopantothenoylcysteine decarboxylase [Danio rerio]
18	gi 47086699	19	25790	1 (1)	1 (1)	0.19 synaptophysin-like 2b [Danio rerio]

$$emPAI = 10^{\frac{N_{observed}}{N_{observable}}} - 1$$

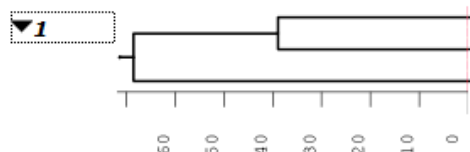
Protein Family Report (Unassigned)



Peptide matches not assigned to protein families (no details means no match)

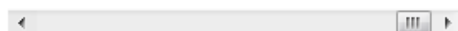
Query	Observed	Mr (expt)	Mr (calc)	Delta	M	Score	Expect	Rank	Peptide
✓103	402.6552	803.2958	803.3960	-0.1002	0	36	0.067	►1	QLDCLR + Carbamidomethyl (C)
✓1471	585.2195	1752.6366	1752.8175	-0.1808	1	32	0.14	►1	AELKQFCDQQLLR + Carbamidomethyl (C); 2 Deamidated (NQ)
✓426	467.6653	933.3161	933.4807	-0.1646	0	31	0.21	►1	LPEAFTEK
✓87	788.1567	787.1495	787.4552	-0.3057	1	31	0.062	►1	TSSLPRK
✓104	402.6621	803.3096	803.3960	-0.0864	0	30	0.28	►1	QLDCLR + Carbamidomethyl (C)
✓724	533.1418	1064.2691	1064.5978	-0.3287	1	29	0.28	►1	STIQKFVSR
✓888	577.1755	1152.3365	1152.5081	-0.1716	0	28	0.36	►1	QMLATGDSTGR + Deamidated (NQ); Oxidation (M)
✓565	495.6717	989.3289	989.4712	-0.1423	1	28	0.39	►1	DAQLQRCR + Deamidated (NQ)
✓1450	579.5521	1735.6344	1735.8967	-0.2623	0	28	0.41	►1	FVLAFSHCITELAGTK
✓872	567.6536	1133.2925	1133.5465	-0.2540	0	27	0.44	►1	DVNQQEFVR
✓1446	868.2705	1734.5265	1734.8498	-0.3233	0	27	0.36	►1	SDILNVFCDNPSLLK + Carbamidomethyl (C); Deamidated (NQ)
✓531	487.1789	972.3433	972.5240	-0.1807	0	27	0.28	►1	LQLQVAGSR + 2 Deamidated (NQ)
✓979	616.6783	1231.3421	1231.5290	-0.1869	1	27	0.25	►1	SGGGGGGGQGNRGGR + 2 Deamidated (NQ)
✓815	553.6602	1105.3059	1105.6131	-0.3072	1	26	0.4	►1	LSKFNLQEK
✓1351	834.0613	1666.1080	1665.8292	0.2788	0	26	0.09	►1	LGMLQPMAMTFLNK + Deamidated (NQ)
✓728	533.1483	1064.2821	1064.5978	-0.3158	1	26	0.41	►1	STIQKFVSR
✓924	589.6949	1177.3753	1177.6819	-0.3066	1	26	0.2	►1	FTTALKATGLR
✓154	416.6778	831.3410	831.4338	-0.0928	0	26	0.28	►1	TSPISAEK
✓156	416.6873	831.3599	831.4338	-0.0738	0	26	0.28	►1	TSPISAEK
✓811	553.6521	1105.2896	1105.5404	-0.2508	0	25	0.51	►1	EAIEFINDR
✓265	437.6829	873.3512	873.4192	-0.0679	0	25	0.43	►1	EAVEEAAR
✓673	519.1601	1036.3057	1036.5665	-0.2608	1	25	0.31	►1	LYNTRITR + Deamidated (NQ)
✓530	487.1779	972.3412	972.5603	-0.2192	1	24	0.44	►1	IQILSKNR + 2 Deamidated (NQ)
✓757	541.1457	1080.2769	1080.5339	-0.2570	0	24	0.29	►1	FINIQTSQK + 3 Deamidated (NQ)
✓1081	671.6989	1341.3832	1341.6598	-0.2766	1	24	0.16	►1	HKILEDQEMAK + Deamidated (NQ)
✓725	533.1423	1064.2700	1064.5978	-0.3279	1	24	0.69	►1	STIQKFVSR
✓135	413.6694	825.3242	825.4457	-0.1215	1	24	0.58	►1	SKEGLHR

Protein Family Report



1 gi|94733733
3 gi|166795887
2 gi|156713467

568 novel protein similar to vitellogenin 1 (vg1) [Danio rerio]
310 vitellogenin 1 [Danio rerio]
343 vitellogenin 7 [Danio rerio]



Threshold (0): 0

Cut

		Score	Mass	Matches	Sequences	emPAI	
☒ 1.1	gi 94733733	568	149143	26 (18)	14 (11)	0.54	novel protein similar to vitellogenin 1 (vg1) [Danio rerio]
	▶ 2 same sets of gi 94733733						
☒ 1.2	gi 156713467	343	148739	14 (11)	10 (8)	0.37	vitellogenin 7 [Danio rerio]
	▶ 1 same set of gi 156713467						
☒ 1.3	gi 166795887	310	149453	21 (12)	14 (9)	0.36	vitellogenin 1 [Danio rerio]
	▶ 1 same set of gi 166795887						

Redisplay

All

None

▼36 peptide matches (28 non-duplicate, 8 duplicate)

☒ Auto-fit to window

Query	Dupes	Observed	Mr (expt)	Mr (calc)	Delta M	Score	Expect	Rank	U	1	2	3	Peptide
426		467.6653	933.3161	933.4841	-0.1680	0	28	0.41	▶2				R.IPEITMSK.R + Oxidation (M)
513		481.6614	961.3083	961.4902	-0.1820	0	18	0.61	▶1	U			R.IPEITMSR.R + Oxidation (M)
1032		433.5155	1297.5247	1297.7030	-0.1783	1	30	0.24	▶1	U			R.LEVEWERLPK.I
1042	▶1	652.2027	1302.3908	1302.6568	-0.2660	0	82	1.6e-006	▶1	U			K.AEAGVLGEYPAAR.L
1231		758.2475	1514.4804	1514.7406	-0.2602	0	91	1.9e-007	▶1	U			K.VFGQEVAYVNFDK.T
1288	▶3	532.8208	1595.4406	1595.7627	-0.3221	0	36	0.04	▶1	U			K.GCVEVHSHNAAFIR.N + Carbamidomethyl (C)
1293		800.2584	1598.5023	1598.8225	-0.3203	0	50	7.4e-005	▶1	U			K.VLISATTENTYLMK.L + Oxidation (M)

Protein Family Report

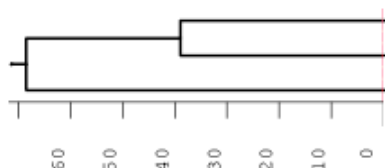


Protein families 1-10 (out of 18)

10 per page 1 [2](#) [Next](#) [Expand all](#) [Collapse all](#)

Accession [Find](#) ☐ Match case

▼1



1 gi|94733733
3 gi|166795887
2 gi|156713467

568 novel protein similar to vitellogenin 1 (vg1) [Danio rerio]
310 vitellogenin 1 [Danio rerio]
343 vitellogenin 7 [Danio rerio]

Threshold (0): 0 [Cut](#)

		Score	Mass	Matches	Sequences	emPAI	
☒	1.1	gi 94733733	568	149143	26 (18)	14 (11)	0.54 novel protein similar to vitellogenin 1 (vg1) [Danio rerio]
	▼2 same sets of gi 94733733						
	gi 125857991	568	148644	26 (18)	14 (11)	0.55	Zgc:136383 protein [Danio rerio]
	gi 160420306	568	148750	26 (18)	14 (11)	0.55	hypothetical protein LOC678536 [Danio rerio]
☒	1.2	gi 156713467	343	148739	14 (11)	10 (8)	0.37 vitellogenin 7 [Danio rerio]
	▼1 same set of gi 156713467						
	gi 94732723	343	147084	13 (11)	9 (8)	0.37	novel protein similar to vitellogenin 1 (vg1) [Danio rerio]
☒	1.3	gi 166795887	310	149453	21 (12)	14 (9)	0.36 vitellogenin 1 [Danio rerio]
	▼1 same set of gi 166795887						
	gi 94733730	310	149140	22 (12)	15 (9)	0.36	vitellogenin 1 [Danio rerio]

[Redisplay](#) [All](#) [None](#)

Protein Family Report



▼36 peptide matches (28 non-duplicate, 8 duplicate)

☒ Auto-fit to window

Query	Dupes	Observed	Mr (expt)	Mr (calc)	Delta M	Score	Expect	Rank	U	1	2	3	Peptide
426		467.6653	933.3161	933.4841	-0.1680	0	28	0.41	2				R.IPEITMSK.R + Oxidation (M)
513		481.6614	961.3083	961.4902	-0.1820	0	18	0.61	1	U			R.IPEITMSR.R + Oxidation (M)
1032		433.5155	1297.5247	1297.7030	-0.1783	1	30	0.24	1	U			R.LEVEWERLPK.I
1042	1	652.2027	1302.3908	1302.6568	-0.2660	0	82	1.6e-006	1	U			K.AEAGVLGEYPAAR.L
1231		758.2475	1514.4804	1514.7406	-0.2602	0	91	1.9e-007	1	U			K.VFGQEVAYVNFDK.T
1288	3	532.8208	1595.4406	1595.7627	-0.3221	0	36	0.04	1	U			K.GCQEVHSHNAAFIR.N + Carbamidomethyl (C)
1293		800.2584	1598.5023	1598.8225	-0.3203	0	50	7.4e-005	1	U			K.VLISATTENTYLMK.L + Oxidation (M)
1335		824.7525	1647.4904	1647.8138	-0.3234	0	88	1.8e-008	1	U			R.VQLIASSIAENDNMK.I + Oxidation (M)
1399	1	571.1982	1710.5727	1710.8876	-0.3149	0	17	0.61	1	U			K.VFAPAGVSPTVMNLHR.G + Oxidation (M)
1401		571.5306	1711.5701	1711.8716	-0.3015	0	7	1.3	3	U			K.VFAPAGVSPTVMNLHR.G + Deamidated (NQ); Oxidation (M)
1453		869.2887	1736.5629	1736.8920	-0.3291	0	45	0.0024	1	U			K.VFAPAGVSPTVMNLYR.G + Oxidation (M)
1536		599.5346	1795.5819	1795.9006	-0.3187	1	17	0.17	1	U			K.HILTAAYDTGFRFER.A
1556		907.1027	1812.1908	1811.8804	0.3104	0	38	0.0012	1	U			K.LMDPLLYEYAGTWPK.D + Oxidation (M)
1583		609.5575	1825.6506	1825.9726	-0.3220	0	47	0.00055	1	U			K.LLGYYLAAYFDKPTAR.V
1584		914.2233	1826.4320	1825.9726	0.4593	0	68	7.4e-007	1	U			K.LLGYYLAAYFDKPTAR.V
1592		612.5411	1834.6014	1834.9002	-0.2988	1	37	0.012	1	U			K.YILNAAAYDTGFRFER.A
1684	1	643.8820	1928.6241	1929.0054	-0.3814	1	40	0.014	1	U			R.ATNSEKEIELTAALPSQK.S
1686	1	965.3224	1928.6302	1929.0054	-0.3752	1	65	8e-006	1	U			R.ATNSEKEIELTAALPSQK.S
1706		653.2248	1956.6525	1957.0116	-0.3590	1	37	0.016	1	U			R.ATNSEKEIELTAALPSQR.S
1726	1	661.5619	1981.6638	1982.0221	-0.3584	1	68	3.9e-005	1	U			R.AEGPAVERLEFEVQVGPR.A
1742		666.8951	1997.6635	1998.0170	-0.3535	1	58	0.00022	1	U			R.AEGPSVERLEFEVQVGPR.A
1799		687.2614	2058.7625	2059.1353	-0.3729	1	31	0.025	1	U			R.LEVEWERLPIIVTTYAK.K
1927		1094.4118	2186.8090	2187.2051	-0.3961	0	47	0.0003	1	U			K.ALQEGIAFQYAKPLAAEVR.R
1928		729.9468	2186.8185	2187.2051	-0.3866	0	84	2.9e-007	1	U			K.ALQEGIAFQYAKPLAAEVR.R
2242		844.6489	2530.9249	2531.3999	-0.4750	1	81	1.7e-007	1	U			K.LTSALAAQLQIPIKFEYANGVVGK.V + Deamidated (NQ)

Protein Family Report



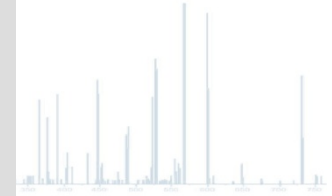
▼36 peptide matches (28 non-duplicate, 8 duplicate)

☒ Auto-fit to window

Query Dups	Observed	Mr (expt)	Mr (calc)	Delta M	Score	Expect	Rank	U	1	2	3	Peptide
426	467.6653	933.3161	933.4841	-0.1680	0	28	0.41	▶2	U	■	■	R.IPEITMSK.R + Oxidation (M)
513	481.6614	961.3083	961.4902	-0.1820	0	18	0.61	▶1	U	■	■	R.IPEITMSR.R + Oxidation (M)
1032	433.5155	1297.5247	1297.7030	-0.1783	1	30	0.24	▶1	U	■	■	R.LEVEWERLPK.I
542: Scan 5770 (rt=1049.07) [D:\LTO ID test\J990504-C1.raw]												
Score > 36 indicates identity												
1042 ▶1	652.2027	1302.3908	1302.6568	-0.2660	0	82	1.6e-006	▶1	U	■	■	K.AEAGVLGEYPAAR.L
Score > 36 indicates identity												
				-0.2449	1	41	0.02	2				+ Carbamidomethyl (C)
				-0.3574	1	22	1.5	3				
				-0.2713	0	18	3.8	4				WNLFWERPR
				-0.2231	1	18	4.1	5				MGWKDGGQGVGPR + Oxidation (M)
				-0.3639	0	13	11	6				ISLNLVGFELAK
				-0.3057	1	13	11	7				ARTVLQMDELK
				-0.1568	0	13	12	8				QAAPNNEWESR + 2 Deamidated (NQ)
				-0.1568	0	13	13	9				QAAPNNEWESR + 2 Deamidated (NQ)
				-0.3361	1	11	18	10				RPRLTHNGPVR + Deamidated (NQ)
1231	758.2475	1514.4804	1514.7406	-0.2602	0	91	1.9e-007	▶1	U	■	■	K.VFGQEVAYVNFDK.T
1288 ▶3	532.8208	1595.4406	1595.7627	-0.3221	0	36	0.04	▶1	U	■	■	K.GCVEVHSHNAAFIR.N + Carbamidomethyl (C)
1293	800.2584	1598.5023	1598.8225	-0.3203	0	50	7.4e-005	▶1	U	■	■	K.VLISATTENTYLMK.L + Oxidation (M)
1335	824.7525	1647.4904	1647.8138	-0.3234	0	88	1.8e-008	▶1	U	■	■	R.VQLIASSIAENDNMK.I + Oxidation (M)
1399 ▶1	571.1982	1710.5727	1710.8876	-0.3149	0	17	0.61	▶1	U	■	■	K.VFAPAGVSPTVMNLHR.G + Oxidation (M)
1401	571.5306	1711.5701	1711.8716	-0.3015	0	7	1.3	▶3	U	■	■	K.VFAPAGVSPTVMNLHR.G + Deamidated (NQ); Oxidation (M)

AJAX (shorthand for asynchronous JavaScript and XML)

New reports – load much more rapidly

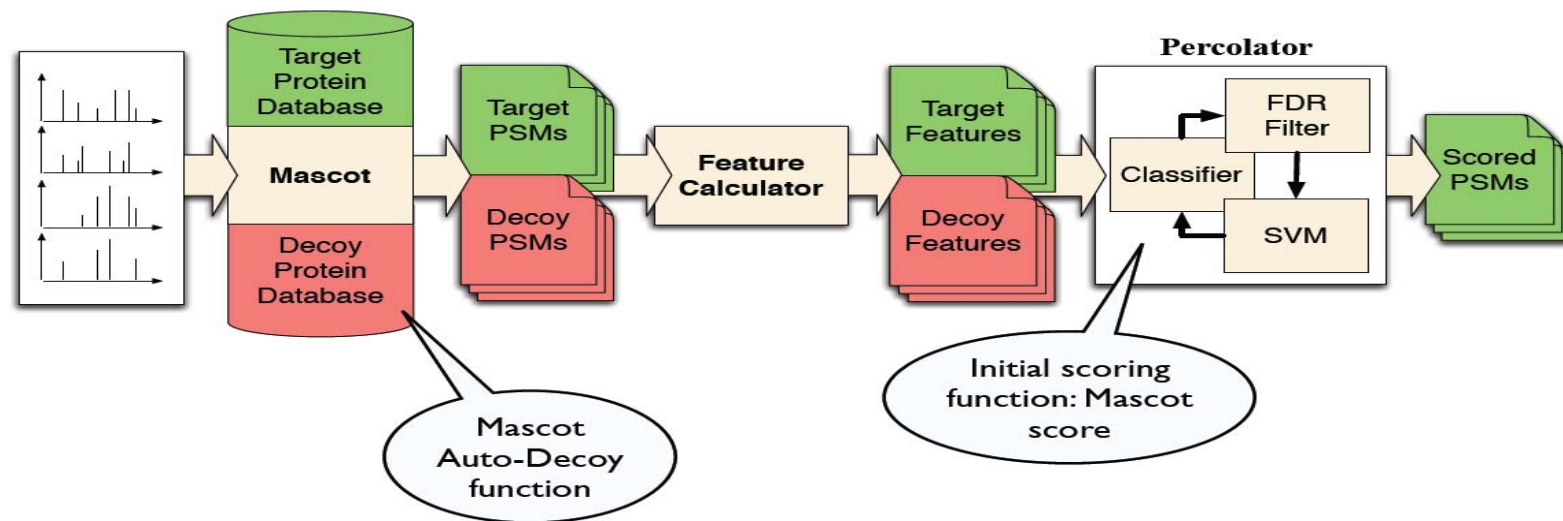


- Search of 114,943 ms-ms spectra against a database with 1,319,480 sequences
- Mascot 2.2:
 - 35 minutes to load report
 - No progress reports
 - 430Mb memory for 1487 proteins
- Mascot 2.3 – dramatic improvement...

Percolator

Percolator is an algorithm that uses semi-supervised machine learning to improve the discrimination **between correct and incorrect spectrum identifications**.

Mascot Percolator



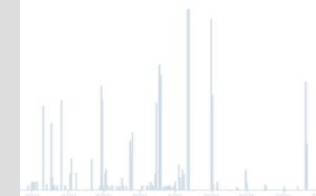
M. Brosch, L. Yu, T. Hubbard, J. Choudhary, *J Proteome Res* (2009).

PSM=Peptide Spectrum Match

FDR=False Discovery Rate

SVM=Support Vector Machine

Quantitation



MATRIX SCIENCE Mascot Search Results

User :
Email :
Search title : SILAC example: NG108-EphB2 from Zhang and Neubert
MS data file : U:\Docs\Quantitation\Multiplex\ga_050905_py1_07.mgf
Database : SwissProt 56.0 (392667 sequences; 141217034 residues)
Quantitation : SILAC K+6 R+6 multiplex [method details](#)
 : Zhang and Neubert, MCP 5 401-411 (2006)
Timestamp : 16 Jul 2009 at 11:30:30 GMT
Protein hits : Heavy/Light

0.072	K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=5
0.080	TRYP_PIG	Trypsin OS=Sus scrofa PE=1 SV=1
---	IGG2B_MOUSE	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3
0.022	ALBU_BOVIN	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
0.752	ENPL_MOUSE	Endoplasmic reticulum protein OS=Mus musculus GN=Hsp90b1 PE=1 SV=2
1.125	NUCL_MOUSE	Nucleolin OS=Mus musculus GN=Ncl PE=1 SV=2
10.512	EPHB2_HUMAN	Ephrin type-B receptor 2 OS=Homo sapiens GN=EPHB2 PE=1 SV=5
0.794	HNRPU_HUMAN	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRPU PE=1 SV=5
1.189	SFPQ_HUMAN	Splicing factor, proline- and glutamine-rich OS=Homo sapiens GN=SFPQ PE=1 SV=2
1.207	CAPR1_MOUSE	Caprin-1 OS=Mus musculus GN=Caprin1 PE=1 SV=2
---	VIME_CRIGR	Vimentin (Fragment) OS=Cricetulus griseus GN=VIM PE=2 SV=1
---	BCAR1_MOUSE	Breast cancer anti-estrogen resistance protein 1 OS=Mus musculus GN=Bcar1 PE=1 SV=1
---	FAK1_MOUSE	Focal adhesion kinase 1 OS=Mus musculus GN=Ptk2 PE=1 SV=3
---	IGKC_MOUSE	Ig kappa chain C region OS=Mus musculus PE=1 SV=1

Quantitation



Peptide Summary Report

Format As	Peptide Summary	Help	
Significance threshold p<	0.05	Max. number of hits	AUTO
Standard scoring ☒ MudPIT scoring ☐	Ions score or expect cut-off	0	Show sub-sets
Show pop-ups ☒ Suppress pop-ups ☐	Sort unassigned	Decreasing Score	Require bold red ☒
Protein ratio type	Weighted	Normalisation	None
Report peptide ratios	☒	Outlier removal	Automatic
Min. precursor charge	1	Peptide threshold	At least homology
Unique peptides only	☐		

Select All

Select None

Search Selected

☐ Error tolerant

1. [K2C1_HUMAN](#) Mass: 65978 Score: 350 Matches: 7(4) Sequences: 6(4)

Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=5

☐ Check to include this hit in error tolerant search

Quantitation:	Ratio	Weighted	N	SD(geo)
	Heavy/Light	0.072	5	1.530

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Heavy/Light	Peptide
☒ 18	590.7374	1179.4603	1178.5931	0.8672	0	54	0.034	1	U	---	K.YEELQITAGR.H
☒ 28	651.7740	1301.5335	1301.7078	-0.1743	0	103	4e-07	1		0.000	R.SLDLDSIIAEVK.A
☒ 33	692.6202	1383.2258	1382.6830	0.5428	0	55	0.02	1	U	0.137	K.SLNNQFASFIDK.V
☒ 38	738.3927	1474.7708	1474.7780	-0.0071	0	74	0.0003	1		0.017	R.FLEQQNQVLQTK.W

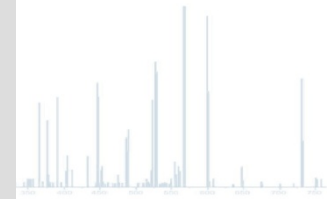
Search Multiple Databases



MASCOT MS/MS Ions Search

Your name	Anna Lizer
Search title	
Database(s)	EST_human contaminants cRAP IPI_arabidopsis IPI_bovine ^ ↓ Allo Quar
Taxonomy	All entries

Select multiple fasta files for searching



- Why:

Best to concatenate a few of your own sequences onto the end of SwissProt or NCBItr

Want to search SwissProt and Trembl, or a species database and contaminants.

- Concatenating fasta files not easy because:

Files are often huge

May need to also update the reference file

Different accession formats

Select multiple fasta files for searching

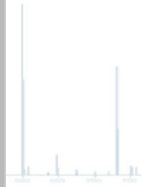


Databases : **1:** SwissProt 51.6
 2: IPI_human 20081014
 (in total 331,981 sequences; 125,104,298 residues)

1.1	1: PPB1 HUMAN	786.8	58259	17	All
	2: IPI00007289	786.8	59938	17	Ta
1.2	1: PPBN HUMAN	605.8	57656	12	All
	2: IPI00290380	605.8	57626	12	Ta
1.3	1: PPBI HUMAN	100.9	57119	2	In
	2: IPI00298622	100.9	57119	2	Ta



鎂陞科技股份有限公司
Mass Solutions Technology



Thanks for your attention

聯絡資訊

鎂陞科技股份有限公司

221台北縣汐止市新台五路一段79號5樓

Tel: 02-26989511; Fax: 02-26989512

info@mass-solutions.com.tw

<http://mass-solutions.com.tw>

