Mascot search (MS/MS Ion Search)

タンパク質試料を酵素消化し、nanoLC-MS/MS装置で測定して得られたペプチドのMS/MSデータと、データベースに登録されているすべてのタンパク質について同じ酵素で消化して得られる仮想ペプチドの理論的なMS/MSデータを照合させて、タンパク質・ペプチドを同定する方法です。
Mascotは、確率的なスコアリングアルゴリズムを採用しているため、統計的に有意なタンパク質・ペプチドをスコアによって明確に区別・可視化することができ、同定タンパク質を容易に抽出することができます。

※参考…Mascot searchによるBSAの解析例（図1,図2）

図1. Mascot Search Results（Peptide Summary Report）

Peptide Summary Report (D:\BSA (std)\20100928\0928 BSA-2.wiff (sample number 1))　　　1/2 ページ

(MATRIX SCIENCE) Mascot Search Results

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User        : JPROS
Email       : pro@jbios.co.jp
Search title: D:\BSA (std)\20100928\0928 BSA-2.wiff (sample number 1)
MS data file: C:\DOCUME~1\ADMINI~1\LOCALS~1\Temp\mas7F.tmp
Database    : NCBInr 20100918 (11833178 sequences; 4040378175 residues)
Taxonomy    : Other mammalia (241831 sequences)
Timestamp   : 28 Sep 2010 at 04:28:53 GMT
Protein hits: gi|1351907   RecName: Full=Serum albumin; AltName: Full=BSA; AltName: Allergen=Bos d 6; Flags: Precursor
              gi|229552    albumin
              gi|193085052 albumin precursor [Capra hircus]
              gi|50953792  serum albumin precursor [Elephas maximus]
```

使用したデータベース：NCBInr
生物種(カテゴリー)とエントリー数：
other mammalia, 241831

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 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.

統計的に有意なイオンスコアの閾値を示します
（有意水準を5%とした場合、このサンプルに関してはイオンスコア38以上）。

Peptide Summary Report

検索する生物種(カテゴリー)の範囲によって、scoringのデフォルトが自動的に変わります。

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 0
Show pop-ups ●　Suppress pop-ups ○　Sort unassigned Decreasing Score　　Require bold red ☐

Select All　　Select None　　Search Selected　　☐ Error tolerant　　Archive Report

1. gi|1351907　Mass: 69248　Score: 371　Queries matched: 39　emPAI: 1.70
   RecName: Full=Serum albumin; AltName: Full=BSA; AltName: Allergen=Bos d 6; Flags: Precursor
   ☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
424	424.2605	846.5064	846.4963	0.0101	1	(12)	48	4	R.LSQKFPK.A
425	424.2936	846.5726	846.4963	0.0763	1	13	33	1	R.LSQKFPK.A
588	450.2512	898.4879	898.4582	0.0296	0	12	38	1	R.LCVLHEK.T + Carboxymethyl (C)
639	461.7656	921.5167	921.4807	0.0359	0	17	12	1	K.AEFVEVTK.L
643	464.2524	926.4902	926.4861	0.0040	0	(17)	12	1	K.YLYEIAR.R
644	464.2589	926.5033	926.4861	0.0172	0	21	4.6	1	K.YLYEIAR.R
646	464.2658	926.5170	926.4861	0.0309	0	(6)	1.4e+002	2	K.YLYEIAR.R
732	487.7516	973.4886	973.4505	0.0381	0	8	1e+002	10	K.DLGEEHFK.G
761	501.8156	1001.6167	1001.5757	0.0410	0	8	1.1e+002	5	K.LVVSTQTALA.-
842	537.2679	1072.5212	1072.4859	0.0353	0	60	0.00069	1	K.SHCIAEVEK.D + Carboxymethyl (C)
864	554.7683	1107.5219	1107.4906	0.0313	0	21	5	1	K.FACFAVEGPK.L + Carboxymethyl (C)

図 2. Protein View

(MATRIX SCIENCE) **Mascot Search Results**

Protein View

Match to: gi|1351907 Score: 371
RecName: Full=Serum albumin; AltName: Full=BSA; AltName: Allergen=Bos d 6; Flags: Precursor
Found in search of C:\DOCUME~1\ADMINI~1\LOCALS~1\Temp\mas7F.tmp

Nominal mass (M_r): 69248; Calculated pI value: 5.82
NCBI BLAST search of gi|1351907 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Bos taurus
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|162648 from Bos taurus

Variable modifications: Carboxymethyl (C),Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 54%

Matched peptides shown in **Bold Red**

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  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA
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[Show predicted peptides also]

[Sort Peptides By]　● Residue Number　○ Increasing Mass　○ Decreasing Mass

Start – End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
35 – 44	417.2223	1248.6449	1248.6139	0.0311	1	R.FKDLGEEHFK.G (Ions score 36)
37 – 44	487.7516	973.4886	973.4505	0.0381	0	K.DLGEEHFK.G (Ions score 8)
66 – 75	582.3200	1162.6255	1162.6234	0.0022	0	K.LVNELTEFAK.T (Ions score 58)
66 – 75	582.3208	1162.6270	1162.6234	0.0037	0	K.LVNELTEFAK.T (Ions score 15)
66 – 75	582.3434	1162.6722	1162.6234	0.0488	0	K.LVNELTEFAK.T (Ions score 8)
76 – 88	489.2043	1464.5911	1464.5497	0.0414	0	K.TCVADESHAGCEK.S 2 Carboxymethyl (C) (Ions score 49)
89 – 100	474.2391	1419.6954	1419.6704	0.0250	0	K.SLHTLFGDELCK.V Carboxymethyl (C) (Ions score 54)
106 – 117	740.7553	1479.4960	1479.4840	0.0120	0	R.ETYGDMADCCEK.Q 2 Carboxymethyl (C) (Ions score 24)
123 – 138	476.4875	1901.9207	1901.8465	0.0742	1	R.NECFLSHKDDSPDLPK.L Carboxymethyl (C) (Ions score 29)
161 – 167	464.2524	926.4902	926.4861	0.0040	0	K.YLYEIAR.R (Ions score 17)
161 – 167	464.2589	926.5033	926.4861	0.0172	0	K.YLYEIAR.R (Ions score 21)
161 – 167	464.2658	926.5170	926.4861	0.0309	0	K.YLYEIAR.R (Ions score 6)
242 – 248	424.2605	846.5064	846.4963	0.0101	1	R.LSQKFPK.A (Ions score 12)
242 – 248	424.2936	846.5726	846.4963	0.0763	1	R.LSQKFPK.A (Ions score 13)
249 – 256	461.7656	921.5167	921.4807	0.0359	0	K.AEFVEVTK.L (Ions score 17)
264 – 280	529.9783	2115.8840	2115.8296	0.0544	1	K.VHKECCHGDLLECADDR.A 3 Carboxymethyl (C) (Ions score 20)
286 – 297	722.8311	1443.6477	1443.6188	0.0290	0	K.YICDNQDTISSK.L Carboxymethyl (C) (Ions score 35)
298 – 309	512.2752	1533.8037	1533.7418	0.0619	1	K.LKECCDKPLLEK.S 2 Carboxymethyl (C) (Ions score 47)
310 – 318	537.2679	1072.5212	1072.4859	0.0353	0	K.SHCIAEVEK.D Carboxymethyl (C) (Ions score 60)
319 – 340	820.3788	2458.1145	2458.1573	-0.0429	1	K.DAIPENLPPLTADFAEDKDVCK.N Carboxymethyl (C) (Ions score 31)
347 – 359	784.3655	1566.7164	1566.7354	-0.0190	0	K.DAFLGSFLYEYSR.R (Ions score 73)
360 – 371	480.6306	1438.8701	1438.8045	0.0656	1	R.RHPEYAVSVLLR.L (Ions score 42)

同定されたペプチドが赤字で表示されます。

各々のシークエンスの Ion scoreが表示されます。

※　解析結果の見方について、ご不明の点がありましたらお問い合わせください。