Experiment 7: Mascot Database Search Using Public Mascot Server

- **General practice for Mascot search**

  BSA emPAI FINAL.tmp

- **emPAI quantitation:**

  BSA emPAI FINAL.tmp

- **iTRAQ quantitation:**

  iTRAQ_DDAcs4b.pkl

- **Error tolerant search:**

  TIF acetylation.tmp

Mascot Search

http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=MIS

MASCOT MS/MS Ions Search

Play with search parameters

Your name	Sheng	Email	sz14@cornell.edu
Search title	Test		
Database	SwissProt		
Taxonomy	. . . . . Mammalia (mammals)		
Enzyme	Trypsin	Allow up to	1 missed cleavages
Fixed modifications	Biotin (K) Biotin (N-term) Carbamidomethyl (C) Carbamyl (K) Carbamyl (N-term)	Variable modifications	NIPCAM (C) Oxidation (HW) Oxidation (M) Phospho (ST) Phospho (Y)
Quantitation	None		
Peptide tol. ±	1.5 Da # ^{13}C 0	MS/MS tol. ±	0.8 Da
Peptide charge	2+ and 3+	Monoisotopic	Average
Data file	C:\Docum and Settings\sz Browse...		
Data format	Mascot generic	Precursor	m/z
Instrument	Default	Error tolerant	
Decoy		Report top	AUTO hits
	Start Search ...		Reset Form

Load BSA…tmp file here

Relative Quantitation: emPAI Protocol

Mascot Search Results

```
User                    : Sheng
Email                   : sz14@cornell.edu
Search title            : Test
MS data file            : C:\Documents and Settings\sz14\My Documents\Proteome1\Cornell27Jan09\Presentations\MS worksho
Database                : SwissProt 57.8 (509019 sequences; 178948533 residues)
Taxonomy                : Mammalia (mammals) (64853 sequences)
Timestamp               : 16 Oct 2009 at 03:27:58 GMT
Enzyme                  : Trypsin
Fixed modifications     : Carbamidomethyl (C)
Variable modifications  : Oxidation (M)
Mass values             : Monoisotopic
Protein Mass            : Unrestricted
Peptide Mass Tolerance  : ± 1.5 Da
Fragment Mass Tolerance: ± 0.8 Da
Max Missed Cleavages    : 1
Instrument type         : Default
Number of queries       : 318
Protein hits            : TRFL_BOVIN   Lactotransferrin OS=Bos taurus GN=LTF PE=1 SV=2
                          ALBU_BOVIN   Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
                          TRYP_PIG     Trypsin OS=Sus scrofa PE=1 SV=1
                          RNAS4_BOVIN  Ribonuclease 4 OS=Bos taurus GN=RNASE4 PE=1 SV=4
```

Play with the filters

Select Summary Report

Format As	Select Summary (protein hits)		Help
	Significance threshold p< 0.01	Max. number of hits AUTO	
	Standard scoring ○ MudPIT scoring ◉	Ions score or expect cut-off 25	Show sub-sets 0
	Show pop-ups ◉ Suppress pop-ups ○	Sort unassigned Decreasing Score	Require bold red ☑

1. TRFL_BOVIN Mass: [illegible] Score: 404 Queries matched: 34 emPAI: 1.32
   Lactotransferrin OS=Bos taurus GN=LTF PE=1 SV=2

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
2	374.2787	746.5428	746.3963	0.1465	0	35	0.21	1	R.APVDAFK.E
50	439.8468	877.6790	877.4658	0.2133	0	43	0.025	1	K.DSALGFLR.I

Relative Quantitation: emPAI Protocol

Format As | Select Summary (protein hits) | Help

Significance threshold p< 0.01 | Max. number of hits AUTO

Standard scoring ○ MudPIT scoring ◉ | Ions score or expect cut-off 25 | Show sub-sets 0

Show pop-ups ◉ Suppress pop-ups ○ | Sort unassigned Decreasing Score | Require bold red ☑

1. TRFL_BOVIN Mass: 80002 Score: 404 Queries matched: 34 emPAI: 1.32
Lactotransferrin OS=Bos taurus GN=LTF PE=1 SV=2

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
2	374.2787	746.5428	746.3963	0.1465	0	35	0.21	1	R.APVDAFK.E
50	439.8468	877.6790	877.4658	0.2133	0	43	0.025	1	K.DSALGFLR.I
70	474.2959	946.5772	946.4906	0.0866	0	43	0.026	1	R.LLCLDGTR.K 71
84	490.2879	978.5612	978.4957	0.0656	0	37	0.084	1	R.AFALECIR.A
107	537.3104	1072.6062	1072.5699	0.0363	0	60	0.00057	1	K.LGAPSITCVR.R 108
112	540.8253	1079.6360	1079.5611	0.0749	0	57	0.00079	1	K.VDSAVLGSR.Y 110 111

2. ALBU_BOVIN Mass: 71244 Score: 157 Queries matched: 13 emPAI: 0.37
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	379.7842	757.5539	757.4156	0.1383	0	36	0.15	1	K.GACLLPK.I
16	395.3147	788.6148	788.4644	0.1504	0	49	0.0088	1	K.LVTDLTK.V
58	449.7627	897.5109	897.4742	0.0367	0	33	0.21	1	R.LCVLHEK.T
65	462.2817	922.5489	921.4807	1.0682	0	36	0.11	2	K.AEFVEVTK.L
67	464.3096	926.6047	926.4861	0.1185	0	36	0.12	1	K.YLYEIAR.R
95	507.8840	1013.7535	1013.6121	0.1414	0	55	0.0016	1	K.QTALVELLK.H
123	569.7853	1137.5561	1137.4907	0.0654	0	59	0.00057	1	K.CCTESLVNR.R
129	582.3986	1162.7826	1162.6234	0.1593	0	48	0.0063	1	K.LVNELTEFAK.T
152	417.3001	1248.8785	1248.6139	0.2647	1	37	0.088	1	R.FKDLGEEHFK.G
162	653.4246	1304.8346	1304.7088	0.1258	0	48	0.0068	1	K.HLVDEPQNLIK.Q

Proteins	emPAI	% Molar	Expected ratio
Lactotransferrin	1.32	78.1	75%
BSA	0.37	21.9	25%
Σ[emPAI]	**1.69**	**100**	

Mascot Quantitation Search for iTRAQ Samples

MASCOT MS/MS Ions Search

Your name	Sheng	Email	sz14@cornell.edu
Search title	Test		
Database	SwissProt		
Taxonomy	All entries		
Enzyme	Trypsin	Allow up to	1 missed cleavages
Fixed modifications	Met->Hsl (C-term M) Methyl (C-term) Methyl (DE) Methylthio (C) mTRAQ (K)	Variable modifications	iTRAQ4plex (K) iTRAQ4plex (N-term) iTRAQ4plex (Y) iTRAQ8plex (K) iTRAQ8plex (N-term)
Quantitation	iTRAQ 4plex		
Peptide tol. ±	0.2 Da # ^{13}C 0	MS/MS tol. ±	0.1 Da
Peptide charge	2+ and 3+	Monoisotopic	Average
Data file	Browse...		
Data format	Micromass (.PKL)	Precursor	m/z
Instrument	Default	Error tolerant	
Decoy		Report top	AUTO hits
	Start Search ...		Reset Form

Load iTRAQ.pkl file here

Quantitation Searching for ITRAQ Labeling

Mascot Search Results

You will get 7 distinct protein hits with 117/114 = 2

```
User            : Sheng
Email           : sz14@cornell.edu
Search title    : Test
MS data file    : C:\Documents and Settings\sz14\My Documents\Proteome1\Cornell27Jan09\Presentations\MS workshop Oct09\
Database        : SwissProt 57.8 (509019 sequences; 178948533 residues)
Quantitation    : iTRAQ 4plex   method details
                : Applied Biosystems iTRAQ(TM) 4-plex
Timestamp       : 14 Oct 2009 at 21:41:22 GMT
Protein hits    : 115/114  116/114  117/114
                  0.135    0.082    2.104   ALBU_BOVIN  Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
                  ---    6195.561   2.077   TRFE_HUMAN  Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=2
                  ---      ---      2.087   LACB_BOVIN  Beta-lactoglobulin OS=Bos taurus GN=LGB PE=1 SV=3
                  ---     0.151     2.174   BGAL_ECOHS  Beta-galactosidase OS=Escherichia coli O9:H4 (strain HS) GN=la
                  ---      ---      2.131   LACB_OVIMU  Beta-lactoglobulin OS=Ovis orientalis musimon GN=LGB PE=1 SV=1
                  ---      ---      2.038   LYSC_CHICK  Lysozyme C OS=Gallus gallus GN=LYZ PE=1 SV=1
                  ---      ---      2.089   BGAL_CITK8  Beta-galactosidase OS=Citrobacter koseri (strain ATCC BAA-895 ,
                  ---      ---      2.200   TRY1_BOVIN  Cationic trypsin OS=Bos taurus PE=1 SV=3
                  ---      ---      2.091   TRFL_HORSE  Lactotransferrin (Fragment) OS=Equus caballus GN=LTF PE=1 SV=1
                  ---      ---      1.996   LALBA_BOSMU Alpha-lactalbumin OS=Bos mutus grunniens GN=LALBA PE=2 SV=1
                  ---      ---       ---    STIP1_BOVIN Stress-induced-phosphoprotein 1 OS=Bos taurus GN=STIP1 PE=2 SV=
                  ---      ---       ---    MYSA_DROME  Myosin heavy chain, muscle OS=Drosophila melanogaster GN=Mhc P
                  ---      ---       ---    ADDB_ANOFW  ATP-dependent helicase/deoxyribonuclease subunit B OS=Anoxybac
```

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 > 43 indicate identity or extensive homology ($p<0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

Quantitation Searching for ITRAQ Labeling

Peptide Summary Report

Play with filters to see what happens

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 ☑
	Protein ratio type Weighted	Normalisation None	Help
	Report peptide ratios ☑	Outlier removal Automatic	Min. # peptides 2
	Min. precursor charge 1	Peptide threshold At least homology	0.05

Select All | Select None | Search Selected | ☐ **Error tolerant**

1. ALBU_BOVIN Mass: 79648 Score: 1224 Queries matched: 46 emPAI: 1.24
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
☐ Check to include this hit in error tolerant search

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.135	2	4.618
	116/114	0.082	10	NN
	117/114	2.104	13	1.043

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	115/114	116/114	117/114	Peptide
☑	67	345.2197	688.4248	688.4353	-0.0105	0	14	42	1	---	---	---	K.VASLR.E
☑	86	403.2510	804.4874	804.4949	-0.0074	0	24	7.6	1	---	---	---	R.ADLAK.Y
	109	449.2468	896.4790	896.4847	-0.0057	0	25	3.5	2	---	---	---	K.AFDEK.L
☑	110	449.2472	896.4798	896.4847	-0.0049	0	(15)	33	1	---	---	---	K.AFDEK.L
☑	126	474.7810	947.5474	947.5531	-0.0057	0	(43)	0.077	1	-0.005	-0.082	1.846	K.TPVSEK.V
☑	127	474.7829	947.5512	947.5531	-0.0019	0	48	0.028	1	-0.063	-0.097	2.271	K.TPVSEK.V
☑	146	492.2636	982.5126	982.5183	-0.0057	0	(29)	1.4	1	---	---	---	R.CASIQK.E

Automatic Error Tolerance Search Results

MASCOT MS/MS Ions Search

Your name	Sheng	Email	sz14@cornell.edu
Search title	Test		
Database	NCBInr		
Taxonomy	. . Archaea (Archaeobacteria)		
Enzyme	Chymotrypsin	Allow up to	2 missed cleavages
Fixed modifications	Biotin (K) Biotin (N-term) Carbamidomethyl (C) Carbamyl (K) Carbamyl (N-term)	Variable modifications	mTRAQ:13C(3)15N(1) (Y) NIPCAM (C) Oxidation (HW) Oxidation (M) Phospho (ST)
Quantitation	None		
Peptide tol. ±	1.5 Da # ^{13}C 0	MS/MS tol. ±	0.8 Da
Peptide charge	2+ and 3+	Monoisotopic	Average
Data file	C:\Documents and Settings\sz Browse...		
Data format	Mascot generic	Precursor	m/z
Instrument	Default	Error tolerant	☑
Decoy	☐	Report top	AUTO hits
	Start Search ...		Reset Form

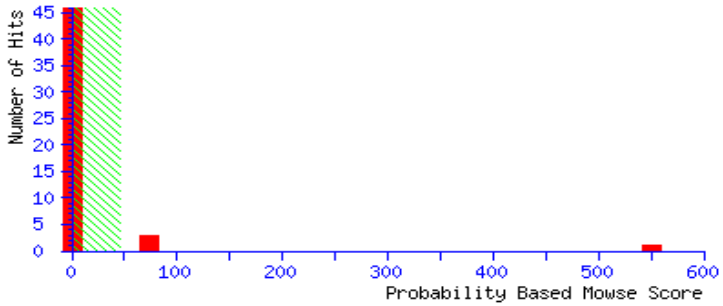
Load acetylation.tmp file here

Automatic Error Tolerance Search Results

```
Email            : sz14@cornell.edu
Search title     : Test
MS data file     : C:\Documents and Settings\sz14\Local Settings\Temp\mas1AB.tmp
Database         : NCBInr 20091010 (9868855 sequences; 3366351629 residues)
Taxonomy         : Archaea (Archaeobacteria) (195008 sequences)
                 : Error tolerant search of all significant protein hits
Timestamp        : 14 Oct 2009 at 20:08:02 GMT
Protein hits     : gi|57641215  transcription initiation factor IIB [Thermococcus kodakarensis KOD1]
                   gi|254170230 TFIIB zinc-binding family [Thermococcus barophilus MP]
                   gi|2392147   Chain B, Tata-Binding ProteinTRANSCRIPTION FACTOR (II)BTATA-Box Complex From Pyro
                   gi|212224249 transcription initiation factor IIB [Thermococcus onnurineus NA1]
```

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 > 45 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Lysine acetylation

Peptide Summary Report

Format As	Peptide Summary		Help
	Significance threshold p< 0.05	Max. number of hits AUTO	Hide error tolerant matches ☐
	Standard scoring ○ MudPIT scoring ◉	Ions score or expect cut-off 30	Show sub-sets 0
	Show pop-ups ◉ Suppress pop-ups ○	Sort unassigned Decreasing Score	Require bold red ☑

Automatic Error Tolerance Search Results

1. gi|57641215 Mass: 34178 Score: 551 Queries matched: 63 emPAI: 2.34
transcription initiation factor IIB [Thermococcus kodakarensis KOD1]

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☑ 26	458.8281	915.6417	915.5025	0.1392	2	60	0.0028	1	F.ALSELDRL.A
☑ 44	506.8177	1011.6209	1011.5713	0.0496	0	46		1	R.ARVGAPESIL.L
☑ 60	537.8238	1073.6331	1073.5717	0.0614	3	55		1	F.ALSELDRLAS.N
☑ 62	538.8439	1075.6733	1075.5509	0.1224	0	47	0.046	1	L.STDIGIDRSL.T
☑ 70	559.3191	1116.6236	1116.5775	0.0461	2	33	1.2	1	L.SELDRLASNL.S
☑ 83	580.3863	1158.7580	1158.6245	0.1336	1	56		1	T.DIGIDRSLTGL.M
☑ 95	603.8331	1205.6517	1205.6291	0.0225	1	46		1	R.RAIEILEEAY.Q
☑ 105	621.9631	1241.9117	1241.6979	0.2137	1	51	0.019	1	L.TSGKSPAGLVAAAL.Y
☑ 118	652.8993	1303.7841	1303.7136	0.0705	2	58		1	T.SGKSPAGLVAAALY.I
☑ 125	665.3942	1328.7738	1328.6936	0.0803	3	60		1	F.ALSELDRLASNL.S + [+27.9949 at S3]
☑ 241	580.7246	1739.1521	1738.9214	0.2307	2	(53)	0.0069	1	L.LHDKGLSTDIGIDRSL.T
☑ 253	594.6780	1781.0123	1780.9683	0.0440	2	60		1	L.LHDKGLSTDIGIDRSL.T + [+42.0470 at K4]
☑ 263	606.4235	1816.2486	1815.9955	0.2532	2	64		1	R.RRAIEILEEAYQRGL.T
☑ 269	920.9549	1839.8953	1839.9690	-0.0737	3	(76)		1	L.LHDKGLSTDIGIDRSLT.G
☑ 270	614.4258	1840.2555	1839.9690	0.2865	3	81		1	L.LHDKGLSTDIGIDRSLT.G
☑ 279	944.8916	1887.7686	1886.9527	0.8159	1	(77)		1	Y.VIEENVVDEGPEWRAF.D + [-0.9476
☑ 280	944.9094	1887.8042	1887.9003	-0.0961	1	(66)	0.00033	1	Y.VIEENVVDEGPEWRAF.D
☑ 281	630.3186	1887.9341	1887.9003	0.0338	1	(40)	0.15	1	Y.VIEENVVDEGPEWRAF.D
☑ 282	630.4111	1888.2115	1887.9003	0.3113	1	(53)	0.0067	1	Y.VIEENVVDEGPEWRAF.D
☑ 283	631.6720	1891.9942	1891.8952	0.0990	1	(73)		1	Y.VIEENVVDEGPEWRAF.D + [+3.9949
☑ 285	949.4550	1896.8955	1896.9905	-0.0950	2	84	5.7e-06	1	L.HDKGLSTDIGIDRSLTGL.M
☑ 287	633.4490	1897.3252	1897.9381	-0.6129	2	(50)		1	L.HDKGLSTDIGIDRSLTGL.M + [+0.9476 at K3]
☑ 288	633.5028	1897.4866	1896.9905	0.4960	2	(69)	0.00018	1	L.HDKGLSTDIGIDRSLTGL.M
☑ 289	633.7361	1898.1864	1896.9905	1.1959	2	(34)	0.59	1	L.HDKGLSTDIGIDRSLTGL.M
☑ 291	635.0363	1902.0871	1901.8795	0.2076	1	(57)		1	Y.VIEENVVDEGPEWRAF.D + [+13.9793 at W13]
☑ 297	639.4067	1915.1983	1914.8968	0.3015	1	43	0.074	1	F.IYDPSRGEIVCKVCGY.V
☑ 299	960.8977	1919.7809	1919.8901	-0.1092	1	(68)		1	Y.VIEENVVDEGPEWRAF.D + [+31.9898 at P11]
☑ 301	642.7319	1925.1739	1924.9854	0.1885	2	(56)		1	L.HDKGLSTDIGIDRSLTGL.M + [+27.9949 at N-term H]
☑ 304	966.8698	1931.7251	1931.9265	-0.2014	1	80		1	Y.VIEENVVDEGPEWRAF.D + [+44.0262 at R14]
☑ 305	645.0688	1932.1845	1931.9265	0.2580	1	(52)		1	Y.VIEENVVDEGPEWRAF.D + [+44.0262 at R14]
☑ 307	970.4502	1938.8859	1939.0375	-0.1515	2	(64)		1	L.HDKGLSTDIGIDRSLTGL.M + [+42.0470 at K3]

Possible Assignments:

Amidine (K) [+41.0265]
Acetyl (K) [+42.0106]
Guanidinyl (K) [+42.0218]
Trimethyl (K) [+42.0469]
Carbamyl (K) [+43.0058]
Schiff Base +43 (KR) [+43.0184]

Automatic Error Tolerance Search Results

Protein View

Match to: gi|57641215 Score: 551
transcription initiation factor IIB [Thermococcus kodakarensis KOD1]
Found in search of C:\Documents and Settings\sz14\Local Settings\Temp\mas1AB.tmp

Nominal mass (M_r): 34178; Calculated pI value: 9.44
NCBI BLAST search of gi|57641215 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Thermococcus kodakarensis KOD1
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|73919861 from Thermococcus kodakarensis
gi|57159539 from Thermococcus kodakarensis KOD1

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Chymotrypsin: cuts C-term side of FYWL unless next residue is P
Sequence Coverage: 58%

Matched peptides shown in Bold Red

```
  1 MSGKRVCPVC GSTEFIYDPS RGEIVCKVCG YVIEENVVDE GPEWRAFDPG
 51 QREKRARVGA PESILLHDKG LSTDIGIDRS LTGLMREKMY RLRKWQSRLR
101 VSDAAERNLA FALSELDRLA SNLSLPKHVE EEAARLYREA VRKGLIRGRS
151 IEAVIAACVY AACRLLKVPR TLDEIADVSR VDKKEIGRSF RFIARHLNLT
201 PKKLFVKPTD YVNKFADELG LSEKVRRRAI EILEEAYQRG LTSGKSPAGL
251 VAAALYIASL MEGEKRTQRE VAEVARVTEV TVRNRYKELV EKLNLKVPIA
301
```

Show predicted peptides also

Sort Peptides By ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Automatic Error Tolerance Search Results

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
16 - 31	639.4067	1915.1983	1914.8968	0.3015	1	F.IYDPSRGEIVCKVCGY.V (Ions score 43)
32 - 44	757.8326	1513.6506	1513.6936	-0.0431	0	Y.VIEENVVDEGPEW.R (Ions score 60)
32 - 44	776.8522	1551.6899	1551.6495	0.0404	0	Y.VIEENVVDEGPEW.R *Cation:K (DE)* [+37.96] (Ions score 52)
32 - 47	944.8916	1887.7686	1886.9527	0.8159	1	Y.VIEENVVDEGPEWRAF.D *Glu->Lys (E)* [-0.95] (Ions score 77)
32 - 47	944.9094	1887.8042	1887.9003	-0.0961	1	Y.VIEENVVDEGPEWRAF.D (Ions score 66)
32 - 47	630.3186	1887.9341	1887.9003	0.0338	1	Y.VIEENVVDEGPEWRAF.D (Ions score 40)
32 - 47	630.4111	1888.2115	1887.9003	0.3113	1	Y.VIEENVVDEGPEWRAF.D (Ions score 53)
32 - 47	631.6720	1891.9942	1891.8952	0.0990	1	Y.VIEENVVDEGPEWRAF.D *Trp->Kynurenin (W)* [+3.99] (Ions score 73)
32 - 47	635.0363	1902.0871	1901.8795	0.2076	1	Y.VIEENVVDEGPEWRAF.D *Trp->Oxolactone (W)* [+13.98] (Ions score 57)
32 - 47	960.8977	1919.7809	1919.8901	-0.1092	1	Y.VIEENVVDEGPEWRAF.D *Dioxidation (P)* [+31.99] (Ions score 68)
32 - 47	966.8698	1931.7251	1931.9265	-0.2014	1	Y.VIEENVVDEGPEWRAF.D *hydroxyethylate (KR)* [+44.03] (Ions score 80)
32 - 47	645.0688	1932.1845	1931.9265	0.2580	1	Y.VIEENVVDEGPEWRAF.D *hydroxyethylate (KR)* [+44.03] (Ions score 52)
56 - 65	506.8177	1011.6209	1011.5713	0.0496	0	R.ARVGAPESIL.L (Ions score 46)
66 - 81	580.7119	1739.1139	1738.9214	0.1925	2	L.LHDKGLSTDIGIDRSL.T (Ions score 45)
66 - 81	580.7246	1739.1521	1738.9214	0.2307	2	L.LHDKGLSTDIGIDRSL.T (Ions score 53)
66 - 81	594.6780	1781.0123	1780.9683	0.0440	2	L.LHDKGLSTDIGIDRSL.T *Trimethyl (K)* [+42.05] (Ions score 60)
66 - 82	920.9549	1839.8953	1839.9690	-0.0737	3	L.LHDKGLSTDIGIDRSLT.G (Ions score 76)
66 - 82	614.4258	1840.2555	1839.9690	0.2865	3	L.LHDKGLSTDIGIDRSLT.G (Ions score 81)
66 - 84	671.1505	2010.4296	2011.0222	-0.5926	3	L.LHDKGLSTDIGIDRSLTGL.M *Lys->Glu (K)* [+0.95] (Ions score 64)
66 - 84	1006.0291	2010.0436	2011.0334	-0.9898	3	L.LHDKGLSTDIGIDRSLTGL.M *Ile->Asn (I)* [+0.96] (Ions score 75)
66 - 84	680.4762	2038.4067	2038.0695	0.3372	3	L.LHDKGLSTDIGIDRSLTGL.M *Formyl (S)* [+27.99] (Ions score 71)
66 - 84	680.4331	2038.2775	2038.1059	0.1716	3	L.LHDKGLSTDIGIDRSLTGL.M *Delta:H(4)C(2) (H)* [+28.03] (Ions score 61)
66 - 84	685.1831	2052.5275	2052.1215	0.4059	3	L.LHDKGLSTDIGIDRSLTGL.M *Trimethyl (K)* [+42.05] (Ions score 74)
67 - 81	813.9027	1625.7908	1625.8373	-0.0465	1	L.HDKGLSTDIGIDRSL.T (Ions score 82)
67 - 81	543.0293	1626.0661	1625.8373	0.2288	1	L.HDKGLSTDIGIDRSL.T (Ions score 53)
67 - 82	576.6852	1727.0338	1726.8850	0.1488	2	L.HDKGLSTDIGIDRSLT.G (Ions score 49)
67 - 84	949.4550	1896.8955	1896.9905	-0.0950	2	L.HDKGLSTDIGIDRSLTGL.M (Ions score 84)
67 - 84	633.5028	1897.4866	1896.9905	0.4960	2	L.HDKGLSTDIGIDRSLTGL.M (Ions score 69)
67 - 84	633.7361	1898.1864	1896.9905	1.1959	2	L.HDKGLSTDIGIDRSLTGL.M (Ions score 34)
67 - 84	633.4490	1897.3252	1897.9381	-0.6129	2	L.HDKGLSTDIGIDRSLTGL.M *Lys->Glu (K)* [+0.95] (Ions score 50)
67 - 84	642.7319	1925.1739	1924.9854	0.1885	2	L.HDKGLSTDIGIDRSLTGL.M *Formyl (N-term)* [+27.99] (Ions score 56)
67 - 84	970.4502	1938.8859	1939.0375	-0.1515	2	L.HDKGLSTDIGIDRSLTGL.M *Trimethyl (K)* [+42.05] (Ions score 64)
72 - 81	538.8439	1075.6733	1075.5509	0.1224	0	L.STDIGIDRSL.T (Ions score 47)
72 - 84	674.4004	1346.7863	1346.7042	0.0822	1	L.STDIGIDRSLTGL.M (Ions score 67)
72 - 84	674.4481	1346.8816	1346.7042	0.1774	1	L.STDIGIDRSLTGL.M (Ions score 34)
74 - 84	580.3863	1158.7580	1158.6245	0.1336	1	T.DIGIDRSLTGL.M (Ions score 56)
112 - 119	458.8281	915.6417	915.5025	0.1392	2	F.ALSELDRL.A (Ions score 60)
112 - 121	537.8238	1073.6331	1073.5717	0.0614	3	F.ALSELDRLAS.N (Ions score 55)
112 - 123	665.3942	1328.7738	1328.6936	0.0803	3	F.ALSELDRLASNL.S *Formyl (S)* [+27.99] (Ions score 60)
114 - 123	559.3191	1116.6236	1116.5775	0.0461	2	L.SELDRLASNL.S (Ions score 33)

Automatic Error Tolerance Search Results

Monoisotopic mass of neutral peptide Mr(calc): 1780.9683
Fixed modifications: Carbamidomethyl (C)
Variable modifications:
K4 : Trimethyl (K)
Ions Score: 60 Expect: 0.00014
Matches (Bold Red): 29/164 fragment ions using 48 most intense peaks

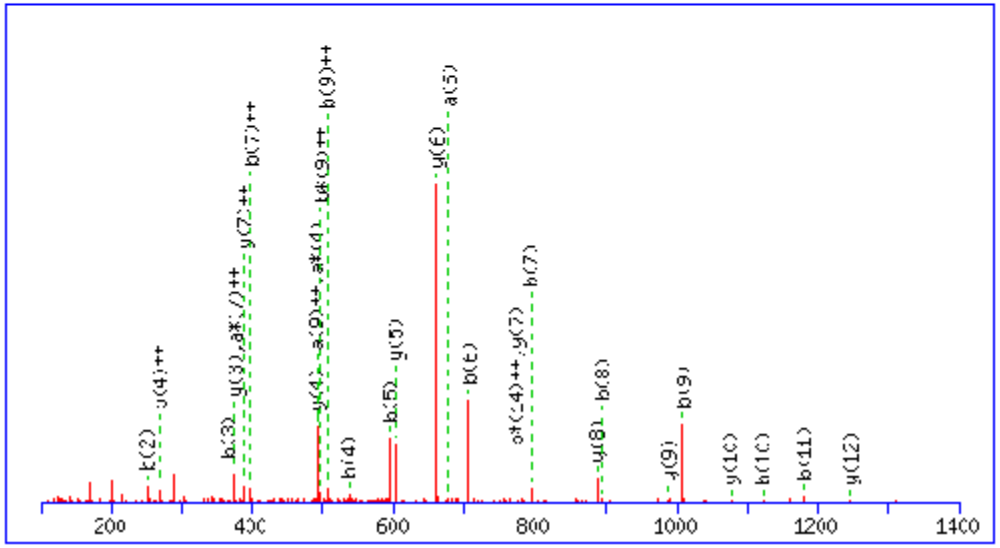
#	a	a++	a*	a*++	b	b++	b*	b*++	Seq.	y	
					114.0913	57.5493			L		
					251.1503	126.0788			H	1668.8915	83
					366.1772	183.5922			D	1531.8326	76
				46.1525	**536.3191**	**268.6632**	519.2926	260.1499	K	1416.8057	70
				74.6632	**593.3406**	297.1739	576.3140	288.6606	G	**1246.6638**	62
				31.2052	**706.4246**	353.7160	689.3981	345.2027	L	1189.6423	59
				74.7212	**793.4567**	**397.2320**	776.4301	388.7187	S	**1076.5582**	53
				25.2451	**894.5043**	447.7558	877.4778	439.2425	T	**989.5262**	49
				82.7585	**1009.5313**	**505.2693**	992.5047	**496.7560**	D	**888.4785**	44
				39.3006	**1122.6154**	561.8113	1105.5888	553.2980	I	**773.4516**	38
				67.8113	**1179.6368**	590.3220	1162.6103	581.8088	G	**660.3675**	33
				24.3533	1292.7209	646.8641	1275.6943	638.3508	I	**603.3461**	30
				81.8668	1407.7478	704.3775	1390.7213	695.8643	D	**490.2620**	24
				59.9174	1563.8489	782.4281	1546.8224	**773.9148**	R	**375.2350**	18
				03.4334	1650.8810	825.9441	1633.8544	817.4308	S	219.1339	11
									L	132.1019	6

Peptide View

MS/MS Fragmentation of **LHDKGLSTDIGIDRSL**
Found in **gi|57641215**, transcription initiation factor IIB [Thermococcus kodakarensis KOD1]

Match to Query 253: 1781.012262 from(594.678030,3+)
Title: File: AcetylBandEMSIDA_11.wiff, Sample: AcetylBandEMSIDA_12 (sample number 1), Eluti
Data file C:\Documents and Settings\sz14\Local Settings\Temp\mas1AB.tmp

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 1400 Full range



Manual Error Tolerance Search Steps

1. Regular search

MASCOT MS/MS Ions Search

Your name: Sheng
Email: sz14@cornell.edu
Search title: Test
Database: NCBInr
Taxonomy: . . Archaea (Archaeobacteria)
Enzyme: Chymotrypsin
Allow up to: 2 missed cleavages
Fixed modifications: Biotin (K), Biotin (N-term), Carbamidomethyl (C), Carbamyl (K), Carbamyl (N-term)
Variable modifications: Oxidation (HW), Oxidation (M), Phospho (ST), Phospho (Y), Propionamide (C)
Quantitation: None
Peptide tol. ±: 1.5 Da
^{13}C: 0
MS/MS tol. ±: 0.8 Da
Peptide charge: 2+ and 3+
Monoisotopic / Average
Data file: C:\Documents and Settings\sz Browse...
Data format: Mascot generic
Precursor: m/z
Instrument: Default
Error tolerant
Decoy
Report top: AUTO hits
Start Search ... Reset Form

2. Manual error tolerance

Mascot Search Results

```
User            : Sheng
Email           : sz14@cornell.edu
Search title    : Test
MS data file    : C:\Documents and Settings\sz14\Local Settings\Temp\mas1
Database        : NCBInr 20091010 (9868855 sequences; 3366351629 residues
Taxonomy        : Archaea (Archaeobacteria) (195008 sequences)
Timestamp       : 14 Oct 2009 at 20:39:49 GMT
Protein hits    : gi|57641215   transcription initiation factor IIB [Therm
                  gi|240102532  Transcription initiation factor TFIIB (tfb
                  gi|254170230  TFIIB zinc-binding family [Thermococcus ba
                  gi|2392147    Chain B, Tata-Binding ProteinTRANSCRIPTION
                  gi|45954      transcription factor IIB [Pyrococcus woese
                  gi|257051641  Transcription factor TFIIB cyclin-related
```

3. Perform search

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 > 45 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

Peptide Summary Report

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 30 — 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

1. gi|57641215 Mass: 34178 Score: 551 Queries matched: 33 emPAI: 2.34
transcription initiation factor IIB [Thermococcus kodakarensis KOD1]
Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
26	458.8281	915.6417	915.5025	0.1392	2	60	0.0028	1	F.ALSELDRL.A
62	538.8439	1075.6733	1075.5509	0.1224	0	47	0.046	1	L.STDIGIDRSL.T

Manual Error Tolerance Search Steps

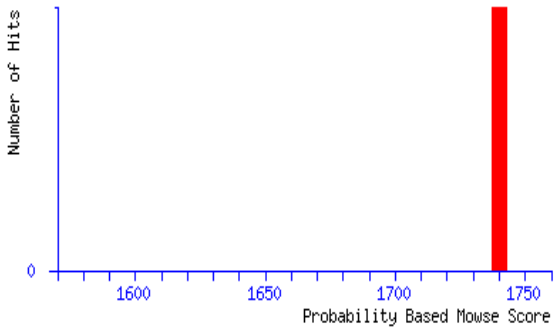
MASCOT MS/MS Ions Search

Your name	Sheng	Email	sz14@cornell.edu
Search title	Test		
Database	NCBInr		
Taxonomy	. . Archaea (Archaeobacteria)		
Enzyme	Chymotrypsin	Allow up to	2 missed cleavages
Fixed modifications	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) aldoamine (R) Amidated (C-term)	Variable modifications	mTRAQ:13C(3)15N(1) (Y) NIPCAM (C) Oxidation (HW) Oxidation (M) Phospho (ST)
Quantitation	None		
Peptide tol. ±	1.5 Da # ^{13}C 0	MS/MS tol. ±	0.8 Da
Peptide charge	2+ and 3+	Monoisotopic	Average
Data file	C:\Documents and Settings\sz14\Local Settings\Temp\mas1AB.tmp		
Data format	Mascot generic	Precursor	m/z
Instrument	Default	Error tolerant search	
		Report top	AUTO hits
	Start Search ...		Reset Form

```
User            : Sheng
Email           : sz14@cornell.edu
Search title    : Test
MS data file    : C:\Documents and Settings\sz14\Local Settings\Temp\mas1AB.tmp
Database        : NCBInr 20091010 (9868855 sequences; 3366351629 residues)
Taxonomy        : Archaea (Archaeobacteria) (195008 sequences)
                : Error tolerant search of 1 entries
Timestamp       : 14 Oct 2009 at 20:47:42 GMT
Protein hits    : gi|57641215 transcription initiation factor IIB [Thermococcus kodaka
```

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



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 35	Show sub-s
	Show pop-ups / Suppress pop-ups	Sort unassigned Decreasing Score	Require bol

Manual Error Tolerance Search Results

1. gi|57641215 Mass: 34178 Score: 1740 Queries matched: 54
transcription initiation factor IIB [Thermococcus kodakarensis KOD1]

☑ Check to include this hit in error tolerant search

Lysine acetylation

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
26	458.8281	915.6417	915.5025	0.1392	2	60		1	F.ALSELDRL.A
62	538.8439	1075.6733	1075.5509	0.1224	0	47		1	L.STDIGIDRSL.T
105	621.9631	1241.9117	1241.6616	0.2501	1	53		1	L.TSGKSPAGLVAAAL.Y + [-0.0364 at K4]
129	674.4004	1346.7863	1346.7042	0.0822	1	67		1	L.STDIGIDRSLTGL.M
134	453.3840	1357.1300	1356.6714	0.4586	2	36		1	L.FVKPTDYVNKF.A + [-0.0364 at K10]
148	703.3965	1404.7784	1404.7249	0.0535	2	60		1	L.TSGKSPAGLVAAALY.I + [-0.0364 at K4]
150	703.4072	1404.7997	1404.7249	0.0749	2	(54)		1	L.TSGKSPAGLVAAALY.I + [-0.0364 at K4]
162	724.4659	1446.9172	1446.7718	0.1454	2	(49)		1	L.TSGKSPAGLVAAALY.I + [+42.0106 at S5]
177	757.8326	1513.6506	1513.6936	-0.0431	0	60		1	Y.VIEENVVDEGPEW.R
187	776.8522	1551.6899	1551.6495	0.0404	0	(52)		1	Y.VIEENVVDEGPEW.R + [+37.9559 at E12]
188	518.3130	1551.9171	1551.6495	0.2676	0	(37)		1	Y.VIEENVVDEGPEW.R + [+37.9559 at C-term W]
206	813.9027	1625.7908	1625.8009	-0.0101	1	82		1	L.HDKGLSTDIGIDRSL.T + [-0.0364 at K3]
240	580.7119	1739.1139	1738.8850	0.2289	2	(45)		1	L.LHDKGLSTDIGIDRSL.T + [-0.0364 at K4]
241	580.7246	1739.1521	1738.8850	0.2671	2	(53)		1	L.LHDKGLSTDIGIDRSL.T + [-0.0364 at K4]
253	594.6780	1781.0123	1780.9683	0.0440	2	60		1	L.LHDKGLSTDIGIDRSL.T + [+42.0470 at K4]
254	594.7963	1781.3672	1780.9683	0.3989	2	(38)		1	L.LHDKGLSTDIGIDRSL.T + [+42.0470 at R14]
265	610.6933	1829.0580	1830.0801	-1.0221	0	(37)		1	L.IRGRSIEAVIAACVY.A + [+2
269	920.9549	1839.8953	1838.9374	0.9579	2	(50)		1	L.LHDKGLSTDIGIDRSL.T + [+
279	944.8916	1887.7686	1886.9527	0.8159	1	(77)		1	Y.VIEENVVDEGPEWRAF.D + [-
280	944.9094	1887.8042	1887.9003	-0.0961	1	(66)		1	Y.VIEENVVDEGPEWRAF.D
281	630.3186	1887.9341	1887.9003	0.0338	1	(40)		1	Y.VIEENVVDEGPEWRAF.D
282	630.4111	1888.2115	1887.9003	0.3113	1	(53)		1	Y.VIEENVVDEGPEWRAF.D
283	631.6720	1891.9942	1891.8952	0.0990	1	(73)		1	Y.VIEENVVDEGPEWRAF.D + [+
285	949.4550	1896.8955	1897.9381	-1.0426	2	84		1	L.HDKGLSTDIGIDRSLTGL.M +
287	633.4490	1897.3252	1897.9381	-0.6129	2	(50)		1	L.HDKGLSTDIGIDRSLTGL.M + [+0.9476 at K3]
288	633.5028	1897.4866	1896.9541	0.5324	2	(69)		1	L.HDKGLSTDIGIDRSLTGL.M + [-0.0364 at K3]
289	633.7361	1898.1864	1897.9494	0.2371	2	(38)		1	L.HDKGLSTDIGIDRSLTGL.M + [+0.9589 at I11]
291	635.0363	1902.0871	1901.8795	0.2076	1	(57)		1	Y.VIEENVVDEGPEWRAF.D + [+13.9793 at W13]
297	639.4067	1915.1983	1914.8604	0.3378	1	43		1	F.IYDPSRGEIVCKVCGY.V + [-0.0364 at K12]
299	960.8977	1919.7809	1919.8901	-0.1092	1	(68)		1	Y.VIEENVVDEGPEWRAF.D + [+31.9898 at P11]
300	963.4504	1924.8862	1925.0218	-0.1356	2	(39)		1	L.HDKGLSTDIGIDRSLTGL.M + 2 [+14.0157 at L15,L18]

Possible Assignments:

Amidine (K) [+41.0265]
Acetyl (K) [+42.0106]
Guanidinyl (K) [+42.0218]
Trimethyl (K) [+42.0469]
Carbamyl (K) [+43.0058]
Schiff Base +43 (KR) [+43.0184]