

How to view Results with Scaffold 3.0

Source: Proteomics Shared Resource,
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Adapted by Protein and Metabolite
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MBB 1.420, 471-2895

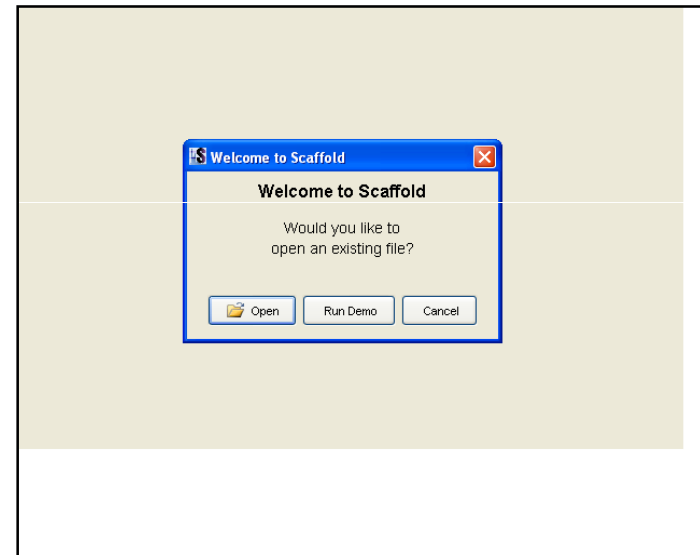
An overview

- This document is intended to walk you through Scaffold version 3.0.
- This is an introductory guide that goes over the basics needed to view your data.
- This guide will skim over several of the more in-depth features of the software.
- If you are interested in learning more about Scaffold you can view their official users guide here:
http://www.proteomesoftware.com/pdf_files/Scaffold3_Users_Guide.pdf or contact PMAF and a member of the lab can sit down with you and go over the software in more detail.

Starting out

- Download Scaffold from http://www.proteomesoftware.com/Proteome_software_prod/Scaffold3_download-main.html
- Follow installation instructions on website, and install normally.
- When the installation is finished double-click on the Scaffold 3.0 icon to begin.
- When prompted to enter a Key select “Free Viewer” to use Scaffold for free to view your data

- Select Open and select the .sfd file containing the data of interest



- The file will load in the viewer (this may take a minute)
- The opened file should be similar to what is on the next page

Scaffold Main Screen

Scaffold Viewer - Samples - LLO933_20110311_VE_Condensed

File Edit View Experiment Export Quant Window Help

Min Protein: 99.0% Min # Peptides: 2 Min Peptide: 95%

Display Options: Unweighted Spectrum Count Req Mods: No Filter Search:

Probability Legend:

- over 95%
- 80% to 94%
- 50% to 79%
- 20% to 49%
- 0% to 19%

MS/MS View: Identified Proteins (136)

#	Protein	Accession Number	Molecular Weight	LLO933_HMELL_20110311_VE	LLO933_KYHIGH_20110311_VE	LLO933_KYLOW_20110311_VE	LLO933_KYMID_20110311_VE	LLO933_TBHIGH_20110311_VE	LLO933_TBLOW_20110311_VE	LLO933_TBHMD_20110311_VE
1	DNA polymerase kappa (POLK_HU...Q9UBT6	Q9UBT6	99 kDa	12	258	695	1107	2		
2	Nuclear factor of activated T-cells...D95644	D95644	101 kDa	1079	5			1		
3	Chaperone protein dnaK (DNAK_E...P0A6Y8	P0A6Y8	69 kDa	3	425	13	3			
4	KERATIN, TYPE II CYTOSKELETAL 1...CONT_088	CONT_088	66 kDa	18	77	60	15	113	56	20
5	KYB-type peptidyl-prolyl cis-tran...P0A090	P0A090	71 kDa		4	33	2	264	42	46
6	keratin 9, cytoskeletal [Homo sap...CONT_068	CONT_068	62 kDa	11	53	58	7	99	38	18
7	TRYPSIN PRECURSOR [Sus scrofa]...CONT_010	CONT_010	24 kDa	8	37	27	10	31	55	45
8	(S43646) cytokeratin 2, CK 2 [hu...CONT_064	CONT_064	66 kDa	3	32	21	2	52	15	1
9	Catabolite gene activator (CRP_E...P0ACJ8	P0ACJ8	24 kDa					14		107
10	keratin 10, type I, cytoskeletal (c...CONT_045	CONT_045	40 kDa	4	23	11		46	14	2
11	keratin 10, type I, epidermal [Ho...CONT_046	CONT_046	57 kDa		18	14	4	29	8	6
12	Dermcidin (DCD_HUMAN). P81605	P81605	11 kDa	0	12	11	2	15	9	9
13	keratin K5, 58K type II, epidermal...CONT_049	CONT_049	62 kDa	1	14	8		26	6	5
14	Promega trypsin artifact 5 K to R...CONT_005	CONT_005	6 kDa	3	8	3		9	13	11
15	[LLO933_sequence] EXTRA_0001	EXTRA_0001	22 kDa					2	44	4
16	Bifunctional polymyxin resistance...C42U97	C42U97	74 kDa					23		20
17	50S ribosomal protein L3 (RL3_EC...C42UH5	C42UH5	22 kDa					26		17
18	keratin, type II cytoskeletal (frag...CONT_080	CONT_080	39 kDa		7	6		15	5	2
19	(L00205) keratin type II [Homo s...CONT_083	CONT_083	60 kDa		4	2		22	5	1
20	30S ribosomal protein S4 (RS4_EC...C42UF1	C42UF1	23 kDa					20	3	12
21	Carbonic anhydrase 2 (CAN_ECOLI).P61517	P61517	25 kDa					8		23
22	50S ribosomal protein L2 (RL2_EC...C42UH2	C42UH2	30 kDa	5				20	3	1
23	Ribose-phosphate pyrophosphoki...P0A717	P0A717	34 kDa	2				6	3	16
24	Succinate dehydrogenase iron-su...P07014	P07014	27 kDa					24		
25	50S ribosomal protein L5 (RL5_EC...C42UG3	C42UG3	20 kDa						27	
26	30S ribosomal protein S3 (RS3_EC...C42UG9	C42UG9	26 kDa					12	4	4
27	50S ribosomal protein L1 (RL1_EC...C5A054	C5A054	25 kDa					23	1	
28	NADH-quinone oxidoreductase su...C42U00	C42U00	25 kDa					16		2
29	KERATIN, TYPE I CYTOSKELETAL 14...CONT_082	CONT_082	52 kDa		4	1		14	3	
30	KADDA_CASEIN DEFOCUSOR TB...CONT_022	CONT_022	21 kDa					46	1	3

Protein Information:

Lookup Accession Number In: NCBI (e.g. |1351907|ALBU_BOV...)

Preferred Accession Number: Q9UBT6

Protein Name: DNA polymerase kappa (POLK_HUMAN).

Sample Information:

Biological Sample:

Sample Category:

Sample Description:

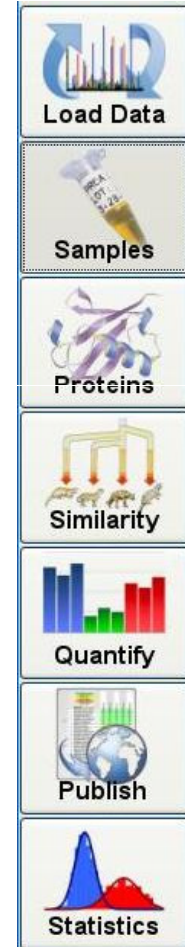
MS/MS Sample:

MS/MS Sample Notes:

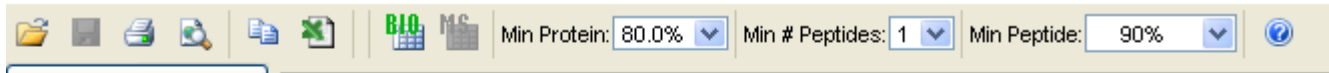
136 Proteins
0.1% Prot %FDR
6538 Spectra
0.3% Pept %FDR

Left Toolbar

- The “Load Data” Tab isn’t used in the free viewer
- “Samples” displays a spread-sheet like format allowing you to sort your data
- “Proteins” shows the MS/MS spectra and % coverage information from a chosen protein
- The “Similarity” tab allows you to sort through proteins with shared peptides.
- “Quantify” gives you access to some basic tools for assessing differences in spectral counts.
- “Publish” creates a paragraph suitable for a methods Section from the settings on the “Samples” page
- The “Statistics” page shows statistical data created from the search algorithm(s) that processed the dataset

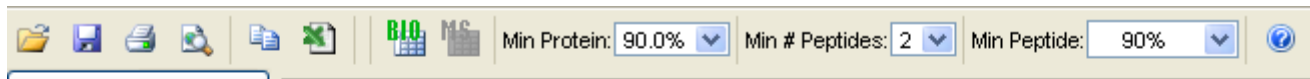


Top Toolbar



- On the far Left of the Top toolbar is the Open, Save, Print, and Print Preview commands respectively (note you can only have one Scaffold file displayed at a time)
- Next are tools for exporting the data to an excel spreadsheet
- “Copy data in current view” copies the displayed data so that you can paste it into an existing excel file and “Export to Excel Spreadsheet” exports all the data and creates a new file.
- The next two tabs switch between viewing the sample’s name and the name of the mass spectrometer raw data file.
- If there are multiple MS/MS samples in the same Biological switching to ‘Biological sample view’ will combine all the results into a single column and ‘MS sample view’ will separate out the samples so that you can see what proteins were identified in each individual MS/MS file.

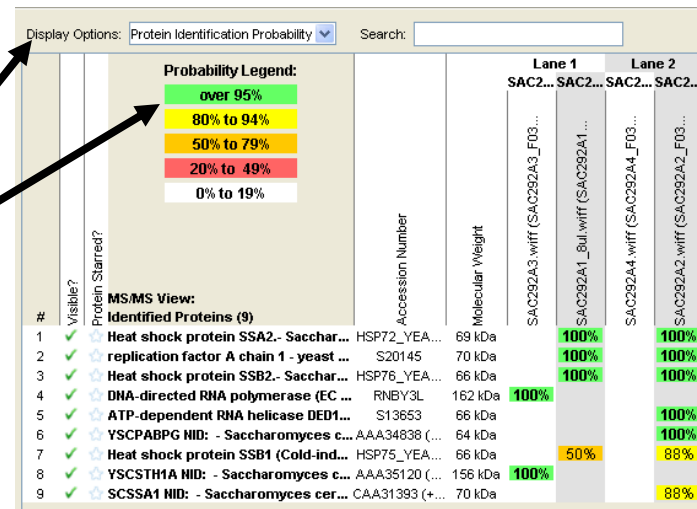
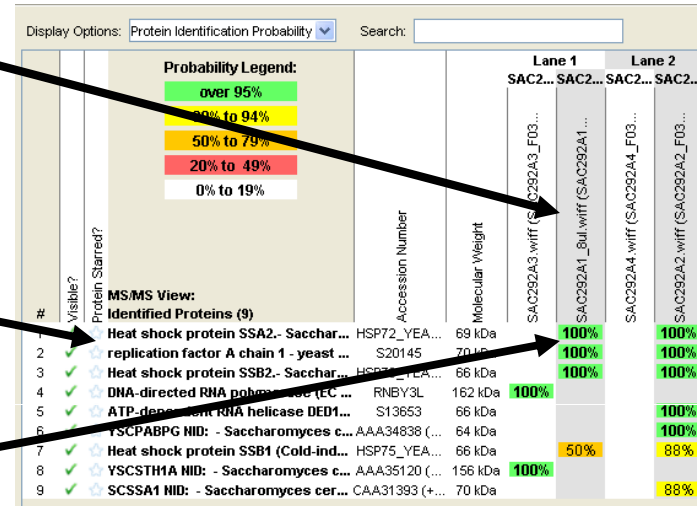
Top Toolbar 2



- “Min Protein,” “Min # of Peptides,” and “Min Peptide” determine what Proteins are displayed on the spreadsheet.
 - Note that Proteins displayed must meet all the criteria listed.
 - So with the criteria above a protein with 2 peptides, each peptide with >90% peptide prob. and 90% protein prob. would be displayed; but a protein with 1 peptide with peptide and protein probabilities of 95% each would not be in the list
- Also note that Protein probability is derived in part from peptide probability, so setting the protein probability much lower than the peptide probability likely won't display any more results
- For more details on how peptide and protein probabilities relate you can view the statistics tab
- The blue “?” access the help menu for scaffold

Samples Screen

- Displays a list of samples on the horizontal axis and proteins on the vertical axis
- Where these columns meet there is a value for that protein in the particular protein
- What value is displayed is determined by the display options tab
- Color corresponds to the Probability Legend referring to Protein Prob.



Display Options

- The Display Options drop-down menu determines what shows up where the two columns intersect
- “Protein Identification Probability” lists the Probability being present
- “% of total spectra” lists what % of the MS/MS spectra were assigned to that protein
- “# of Identified Spectra” gives the total # of spectra assigned to the protein
- “# of Unique Peptides” lists the # of Unique peptides in the identified protein (note that missed cleavages/degradation products are considered a different peptide)
- Finally “# of unique Spectra” is similar to the “# of Identified Spectra” but counts spectra of different charge states, but matching the same amino acid sequence, only once.

Display Options: Protein Identification Probability Search:

Protein Identification Probability
 Percentage of Total Spectra
 Number of Identified Spectra
 Number of Unique Peptides
 Number of Unique Spectra

20% to 49%
 0% to 19%

#	Visible?	Protein Starred?	MS/MS View: Identified Proteins (9)	Accession Number	Molecular Weight	Lane 1		Lane 2	
						SAC2... SAC2...	SAC2... SAC2...	SAC2... SAC2...	SAC2... SAC2...
1	✓	⊛	Heat shock protein SSA2.- Sacchar...	HSP72_YEA...	69 kDa				
2	✓	⊛	replication factor A chain 1 - yeast ...	S20145	70 kDa				
3	✓	⊛	Heat shock protein SSB2.- Sacchar...	HSP76_YEA...	66 kDa				
4	✓	⊛	DNA-directed RNA polymerase (EC ...	RNBY3L	162 kDa	100%			
5	✓	⊛	ATP-dependent RNA helicase DED1...	S13653	66 kDa				
6	✓	⊛	YSCPABPG NID: - Saccharomyces c...	AAA34838 (...)	64 kDa				
7	✓	⊛	Heat shock protein SSB1 (Cold-ind...	HSP75_YEA...	66 kDa		50%		88%
8	✓	⊛	YSCSTH1A NID: - Saccharomyces c...	AAA35120 (...)	156 kDa	100%			
9	✓	⊛	SCSSA1 NID: - Saccharomyces cer...	CAA31393 (+ ...)	70 kDa				88%

Sorting data

- Clicking on any of the horizontal axis columns sorts the data
- Clicking once sorts the data, clicking twice sorts it in the opposite order, and clicking a 3rd time returns the data to its original look

Display Options: Protein Identification Probability Search:

Probability Legend:

- over 95%
- 80% to 94%
- 50% to 79%
- 20% to 49%
- 0% to 19%

#	Visible?	Protein Started?	MS/MS View:	Identified Proteins (9)	Accession Number	Molecular Weight	Lane 1	Lane 2
1	✓	✓	Heat shock protein SSA2- Sacchar...	HSP72_YEA...	69 kDa		100%	100%
2	✓	✓	replication factor A chain 1 - yeast	S20145	70 kDa		100%	100%
3	✓	✓	Heat shock protein SSB2- Sacchar...	HSP76_YEA...	66 kDa		100%	100%
4	✓	✓	DNA-directed RNA polymerase (EC ...	RNBY3L	162 kDa	100%		
5	✓	✓	ATP-dependent RNA helicase DED1...	S13653	66 kDa		100%	100%
6	✓	✓	YSCPABPG NID- Saccharomyces c...	AAA34838 (...)	64 kDa			100%
7	✓	✓	Heat shock protein SSB1 (Cold-ind...	HSP75_YEA...	66 kDa		50%	88%
8	✓	✓	YSCSTH1A NID- Saccharomyces c...	AAA35120 (...)	156 kDa	100%		
9	✓	✓	SCSSA1 NID- Saccharomyces cer...	CAA31393 (+...)	70 kDa			88%

once

Display Options: Protein Identification Probability Search:

Probability Legend:

- over 95%
- 80% to 94%
- 50% to 79%
- 20% to 49%
- 0% to 19%

#	Visible?	Protein Started?	MS/MS View:	Identified Proteins (9)	Accession Number	Molecular Weight	Lane 1	Lane 2
1	✓	✓	YSCPABPG NID- Saccharomyces c...	AAA34838 (...)	64 kDa			100%
2	✓	✓	ATP-dependent RNA helicase DED1...	S13653	66 kDa			100%
3	✓	✓	Heat shock protein SSB2- Sacchar...	HSP76_YEA...	66 kDa	100%		100%
4	✓	✓	Heat shock protein SSB1 (Cold-ind...	HSP75_YEA...	66 kDa	50%		88%
5	✓	✓	Heat shock protein SSA2- Sacchar...	HSP72_YEA...	69 kDa	100%		100%
6	✓	✓	SCSSA1 NID- Saccharomyces cer...	CAA31393 (+...)	70 kDa			88%
7	✓	✓	replication factor A chain 1 - yeast	S20145	70 kDa		100%	100%
8	✓	✓	YSCSTH1A NID- Saccharomyces c...	AAA35120 (...)	156 kDa	100%		
9	✓	✓	DNA-directed RNA polymerase (EC ...	RNBY3L	162 kDa	100%		

Click here

3 times

Display Options: Protein Identification Probability Search:

Probability Legend:

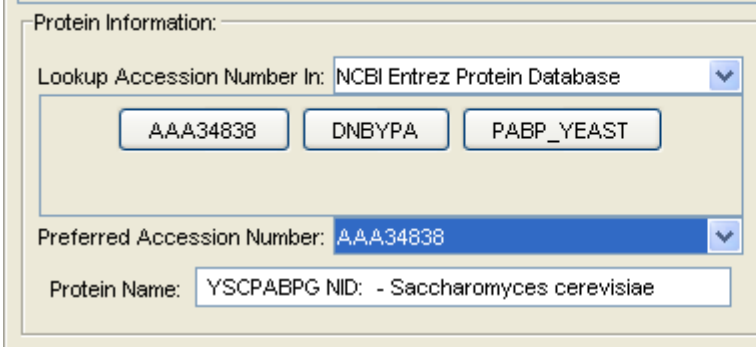
- over 95%
- 80% to 94%
- 50% to 79%
- 20% to 49%
- 0% to 19%

#	Visible?	Protein Started?	MS/MS View:	Identified Proteins (9)	Accession Number	Molecular Weight	Lane 1	Lane 2
1	✓	✓	DNA-directed RNA polymerase (EC ...	RNBY3L	162 kDa	100%		
2	✓	✓	YSCSTH1A NID- Saccharomyces c...	AAA35120 (...)	156 kDa	100%		
3	✓	✓	replication factor A chain 1 - yeast	S20145	70 kDa		100%	100%
4	✓	✓	SCSSA1 NID- Saccharomyces cer...	CAA31393 (+...)	70 kDa			88%
5	✓	✓	Heat shock protein SSA2- Sacchar...	HSP72_YEA...	69 kDa			100%
6	✓	✓	Heat shock protein SSB1 (Cold-ind...	HSP75_YEA...	66 kDa		50%	88%
7	✓	✓	Heat shock protein SSB2- Sacchar...	HSP76_YEA...	66 kDa	100%		100%
8	✓	✓	ATP-dependent RNA helicase DED1...	S13653	66 kDa		100%	100%
9	✓	✓	YSCPABPG NID- Saccharomyces c...	AAA34838 (...)	64 kDa			100%

twice

Other Sample Screen info

- Hovering your mouse over a value in the table will display more details
- When hovered over the Protein name this displays all proteins with which the identified peptides are a strong match. If more than one protein is listed here then you do not have enough sequence information to determine the protein your peptides belong to, but instead have one or more of the proteins listed in your sample.
 - This list can also be viewed under the “Proteins” Tab on the Left Scroll Bar
- At the bottom of the page is a protein information screen. This interface allows you to look up your protein on-line at various sites.
 - This will allow you to find more information on your protein, but what these screens reveal is beyond the scope of this guide to cover



Protein Information:

Lookup Accession Number In: NCBI Entrez Protein Database

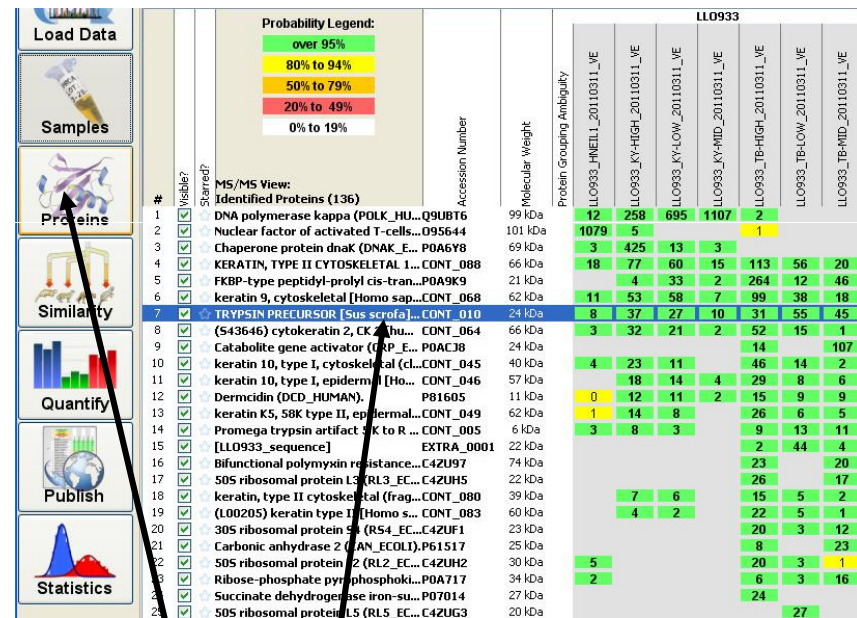
AAA34838 DNBYP A PABP_YEAST

Preferred Accession Number: AAA34838

Protein Name: YSCPABPG NID: - Saccharomyces cerevisiae

Protein Screen

- To view data in the “Proteins” Tab on the left tool bar first select a protein in the “Samples” screen by clicking on it.
- Then select the “Proteins” Tab on the left toolbar



First click here

Then here

Main Protein Screen

File Edit View Experiment Export Quant Window Help

Min Protein: 99.0% Min # Peptides: 2 Min Peptide: 95%

TRYPSIN PRECURSOR [Sus scrofa] (gi13642...)

Sequence Coverage	Protein	Category	Bio Sample	MS/MS Sa...	Prob
100%	TRYPSIN PR...	Uncategoriz...	LL0933	LL0933_TB...	100%
100%	TRYPSIN PR...	Uncategoriz...	LL0933	LL0933_TB...	100%
100%	TRYPSIN PR...	Uncategoriz...	LL0933	LL0933_KY...	100%
100%	TRYPSIN PR...	Uncategoriz...	LL0933	LL0933_KY...	100%
100%	TRYPSIN PR...	Uncategoriz...	LL0933	LL0933_KY...	100%
100%	TRYPSIN PR...	Uncategoriz...	LL0933	LL0933_HN...	100%

Go...	Sequence	Prob	SEQ...	SEQ...	NTT	Modifications
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	2.74	0.21	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	2.74	0.30	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	3.81	0.25	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	3.32	0.30	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	4.00	0.30	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	3.58	0.37	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	4.21	0.47	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	4.59	0.38	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	5.09	0.51	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	5.31	0.51	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	5.27	0.42	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	4.36	0.49	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	5.73	0.43	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	5.73	0.55	2	
✓	(K)IITHPNFNGNTLNDIMLIK(L)	95%	5.33	0.58	2	
✓	(K)IITHPNFNGNTLNDIMLIK(LSPATI)	95%	4.63	0.49	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(LSPATI)	95%	4.63	0.52	2	Oxidation (+16)
✓	(K)IITHPNFNGNTLNDIMLIK(LSPATL)	95%	5.83	0.51	2	
✓	(K)IITHPNFNGNTLNDIMLIK(LSPATL)	95%	5.42	0.65	2	
✓	(R)IQVRLGHNIDVLEGNQFINAAK(I)	95%	7.10	0.59	2	
✓	(R)LGEHNIDVLEGNQFINAAK(I)	95%	2.94	0.33	2	
✓	(R)LGEHNIDVLEGNQFINAAK(I)	95%	4.06	0.27	2	
✓	(R)LGEHNIDVLEGNQFINAAK(I)	95%	3.63	0.33	2	
✓	(R)LGEHNIDVLEGNQFINAAK(I)	95%	4.02	0.32	2	

Protein Sequence Similar Proteins Spectrum Spectrum/Model Error Fragmentation Table

CONT_010 (100%), 24,409.3 Da
 TRYPSIN PRECURSOR [Sus scrofa] (gi136429|sp|P00761|TRYP_PIG).
 5 unique peptides, 8 unique spectra, 45 total spectra, 62/231 amino acids (27% coverage)

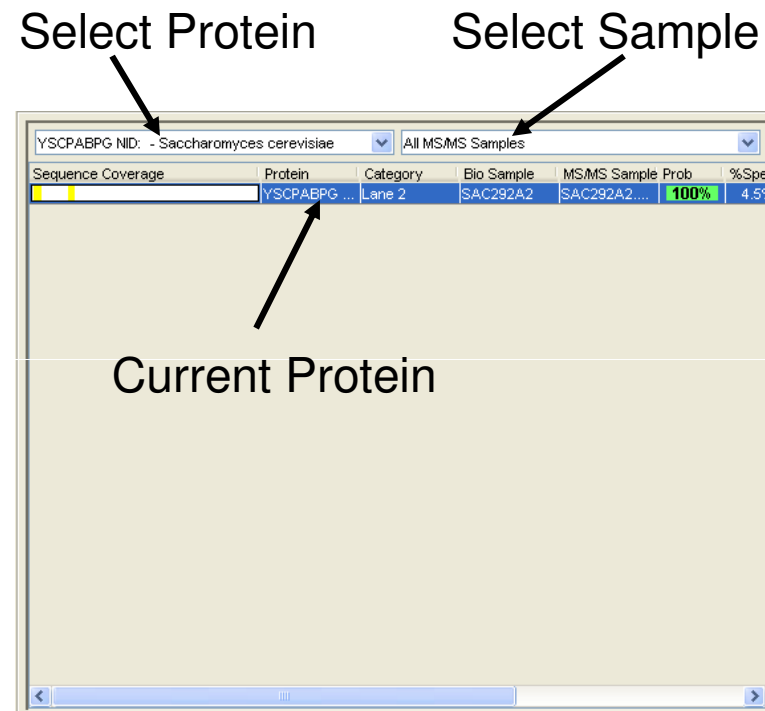
```

F P T D D D D K I V G G Y T C A A N S I P Y Q V S L N S G S H F C G G S L I N S Q W V V S A A H C Y
K S R I Q V R L G E H N I D V L E G N E Q F I N A A K I I T H P N F N G N T L D N D I M L I K L S S
P A T L N S R V A T V S L P R S C A A A G T E C L I S G W G N T K S S G S S Y P S L L Q C L K A P V
L S D S S C K S S Y P G Q I T G N M I C V G F L E G G K D S C Q G D S G G P V V C N G Q L Q G I V S
W G Y G C A Q K N K P G V Y T K V C N Y V N W I Q Q T I A A N
  
```

136 Proteins
 0.1% Prot %FDR
 6538 Spectra
 0.3% Pept %FDR

Upper Left Window

- The Upper left window contains much of the same information as the “Samples” Tab
- The Chosen Protein is listed
- Other Proteins can be chosen from various samples using the drop-down menus



Upper Right Window

- The upper right window displays the peptides which have been assigned to the protein in the upper left window
- Values from the database search are included as well (i.e. mascot identity score)
- Modified amino acids are shown as a green letter in the sequence

Good?	Sequence	Prob	Mascot...	Mascot...	NTT	Modifications	Obser
✓	(K)AIEQLNYTPK(G)	95%	64.4	41.2	2		64
✓	(K)AEGLENLNIQDDGK(G)	95%	89.1	40.6	2		87

Lower window

- The lower window has 6 tabs to display information about the current protein
- The Protein Sequence tab shows the location of identified peptides on the protein
- Amino-acids matched to a MS/MS spectrum are in yellow. Amino-acids marked in green have a post-translational modification (i.e. phosphorylation)
- Hovering the mouse pointer over a yellow amino acid sequence will display a list of all the spectra matching that part of the sequence

Protein Sequence | Similar Proteins | Spectrum | Spectrum/Model Error | Fragmentation Table

S13653 (100%), 65554.5 Da
ATP-dependent RNA helicase DED1 - yeast (*Saccharomyces cerevisiae*)
6 unique peptides, 6 unique spectra, 6 total spectra, 71/604 amino acids (12% coverage)

```
MAELSEQVQN  LSINDNNENG  YVPPHLRGKP  RSARNNSSNY  NNNNGGYNGG
RGGGSFFSNN  RRGGYGNGGF  FGGNNGGSR  NGRSGGRWID  GKHVPAPRNE
KAEIAIFGVP  EDPNFQSSGI  NFDNYDDIPV  DASGKDVPEP  ITEFTSPPLD
GLLLENIKLA  RFTKPTPVQK  YSVPIVANGR  DLMACAQTGS  GKTGGFLFPV
LSESFKTGPS  PQPESQGSFY  QRKAYPTAVI  MAPTRELATQ  IFDEAKKFTY
RSWVKACVVY  GGSPIGNQLR  EIERGCDLLV  ATPGRLNDLL  ERGKISLANV
KYLVLDEADR  MLDMGFEPQI  RHIVEDCDMT  PVGERQTLMF  SATFPADIQH
LARDFLSDYI  FLSVGRVGST  SENITQKVLY  VENQDKKSAL  LDLLSASTDG
LTLIFVETKR  MADQLTDFLI  MQNFRATAIH  GDRTQSERER  ALAAFRSGAA
TLLVATAVAA  RGLDIPNVTH  VINYDLPSDV  DDYVHRIGRT  GRAGNTGLAT
AFFNSENSNI  VKGLHEILTE  ANQEVPSFLK  DAMMSAPGSR  SNSRRGGFGR
NNNRDYRKAG  GASAGGWGSS  RSRDNSFRGG  SGWGSDSKSS  GWGNSGGSSN
SSWW
```

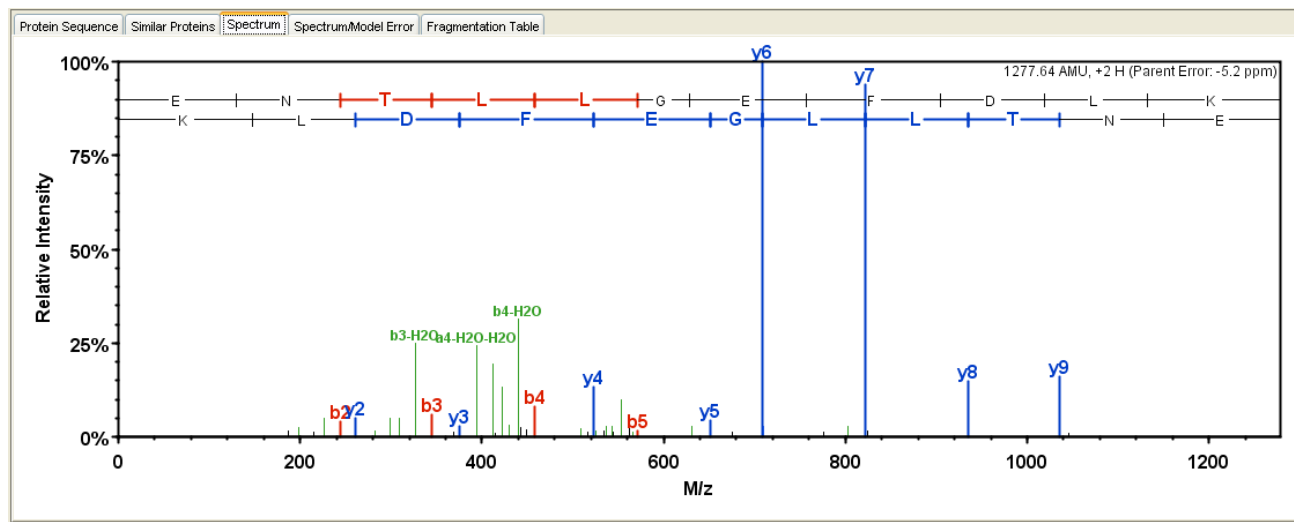

Lower Window 2

- The “similar proteins” tab lists all the protein which share the sequences identified (yellow/green) in the “protein sequence” tab
- If there is more than one protein listed here than there isn’t enough identified sequence information to distinguish between the proteins listed.
- This is common with genes that are heavily processed after transcription (i.e. exons and/or post translational modifications)

Sequence Coverage	Protein	Accession	Prob	%Spec	#Pep	#Uniq	#Spec	%Cov	Weight
	Heat shock protein SSB1 (Cold-inducible protein ...	HSP75_YEAST	50%	0.89%	1	1	1	20%	66454
	dnaK-type molecular chaperone SSB1 - yeast (S...	S20149	50%	0.89%	1	1	1	20%	66585

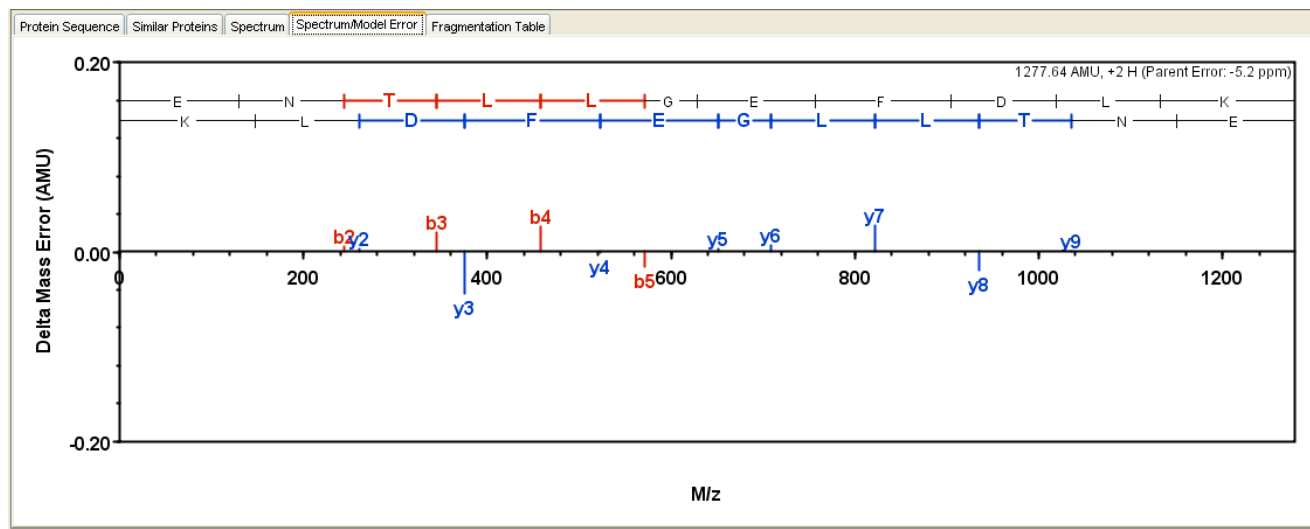
Lower Window 3

- The Spectrum tab displays the MS/MS spectra which the mass spectrometer generated, this is matched against the peptide in the database which lined up best with the fragmentation pattern
- B-ion and y-ion series are color-coded (red and blue) and the amino-acid sequence is across the top, and the parent ion mass is listed.
- Please note that this is a graphical representation and will differ in appearance slightly from the actual MS/MS spectra generated by the mass spectrometer



Lower Window 4

- This window displays the Spectrum/Model error
- The bars on the graph show how far the masses recorded by the mass spectrometer differ from the calculated masses.
- When a spectrum and peptide are matched correctly the error for the peaks should match up well to the mass accuracy of the mass spectrometer used.



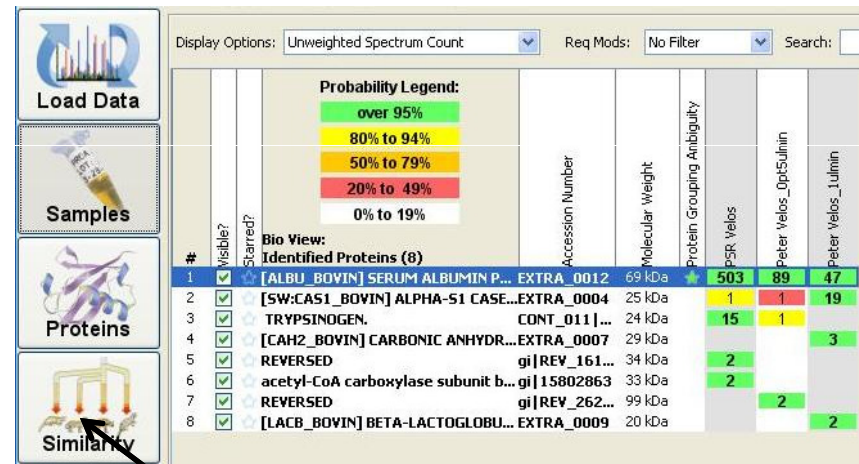
Lower Window 5

- The fragmentation table displays the same information as the spectrum window, but in a spreadsheet format.
- Potential ions which match the spectra are colored (these colored boxes are the lines in the spectra window)
- Green boxes refer to neutral loss or similar fragmentation patterns; this is the same as the green bars in the spectrum window

Protein Sequence											Similar Proteins											Spectrum											Spectrum/Model Error											Fragmentation Table										
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y																																												
1	130.1			112.0	E	1278.7	639.8	1261.6	1260.7	11																																												
2	244.1		227.1	226.1	H	1149.6	575.3	1132.6	1131.6	10																																												
3	345.1		328.1	327.1	T	1035.6	518.3	1018.6	1017.6	9																																												
4	458.2		441.2	440.2	L	934.5	467.8	917.5	916.5	8																																												
5	571.3		554.3	553.3	L	821.4	411.2	804.4	803.4	7																																												
6	628.3	314.7	611.3	610.3	G	708.4	354.7	691.3	690.4	6																																												
7	757.4	379.2	740.4	739.4	E	651.3		634.3	633.3	5																																												
8	904.4	452.7	887.4	886.4	F	522.3		505.3	504.3	4																																												
9	1019.5	510.2	1002.4	1001.5	D	375.2		358.2	357.2	3																																												
10	1132.5	566.8	1115.5	1114.5	L	260.2		243.2		2																																												
11	1278.7	639.8	1261.6	1260.7	K	147.1		130.1		1																																												

Protein Similarity

- A red star in the Protein Grouping Ambiguity means there are proteins that have shared peptides that haven't been examined in the similarity tab yet.
- Selecting that protein and clicking on the similarity tab will allow you to sort through the peptides.



Display Options: Unweighted Spectrum Count Req Mods: No Filter Search:

Probability Legend:

- over 95%
- 80% to 94%
- 50% to 79%
- 20% to 49%
- 0% to 19%

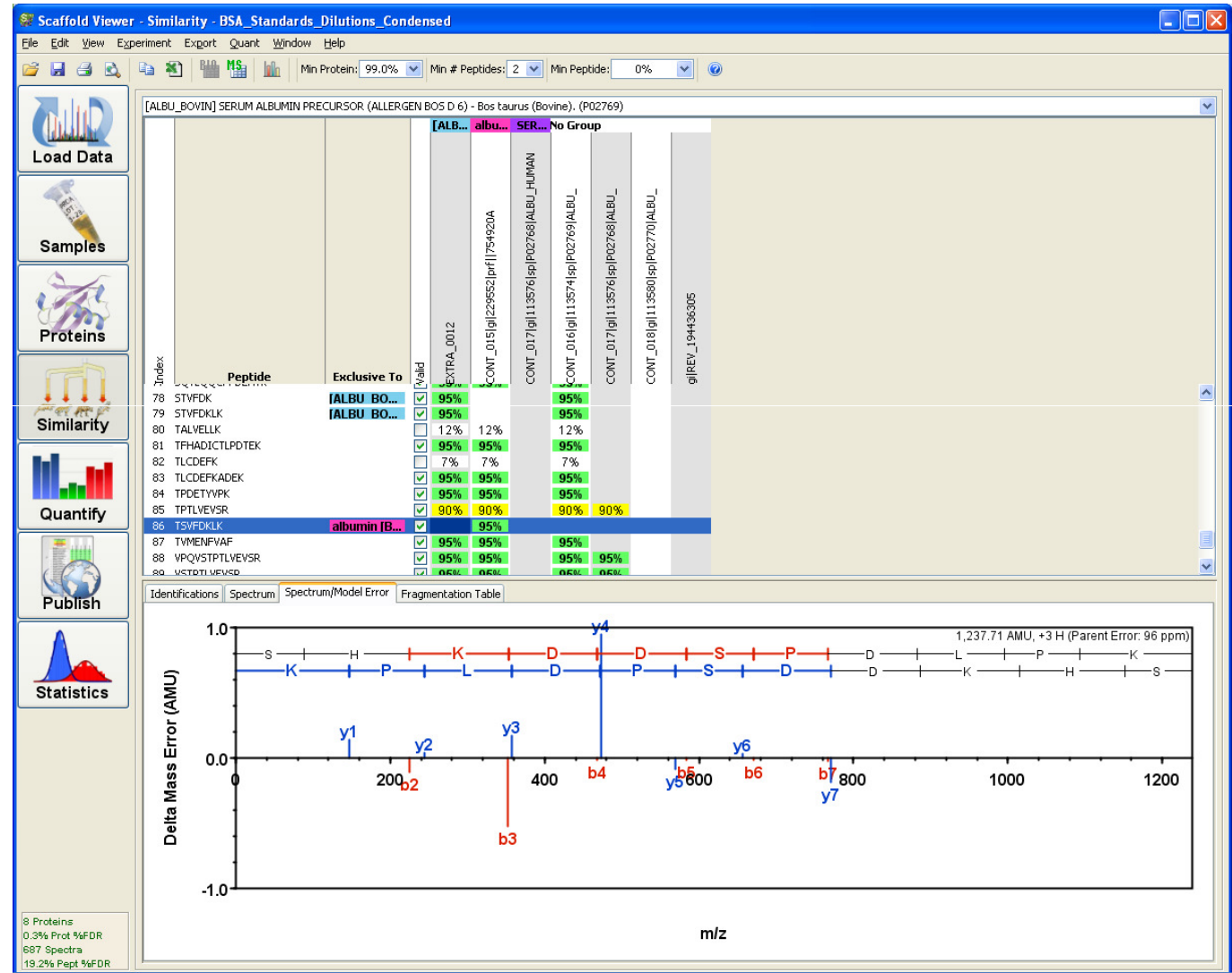
Bio View: Identified Proteins (8)

#	Visible?	Starred?	Accession Number	Molecular Weight	Protein Grouping Ambiguity	PSM Velos	Peter Velos_OppSulfinin	Peter Velos_LuInin
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	[ALBU_BOVIN] SERUM ALBUMIN P... EXTRA_0012	69 kDa	★	503	89	47
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	[SW:CAS1_BOVIN] ALPHA-S1 CASE...EXTRA_0004	25 kDa		1	1	19
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	TRYPsinogen. CONT_011 ...	24 kDa		15	1	
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	[CAH2_BOVIN] CARBONIC ANHYDR...EXTRA_0007	29 kDa				3
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	REVERSED gi REV_161...	34 kDa		2		
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	acetyl-CoA carboxylase subunit b... gi 15802863	33 kDa		2		
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	REVERSED gi REV_262...	99 kDa			2	
8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	[LACB_BOVIN] BETA-LACTOGLOBU... EXTRA_0009	20 kDa				2

Select the protein and click here

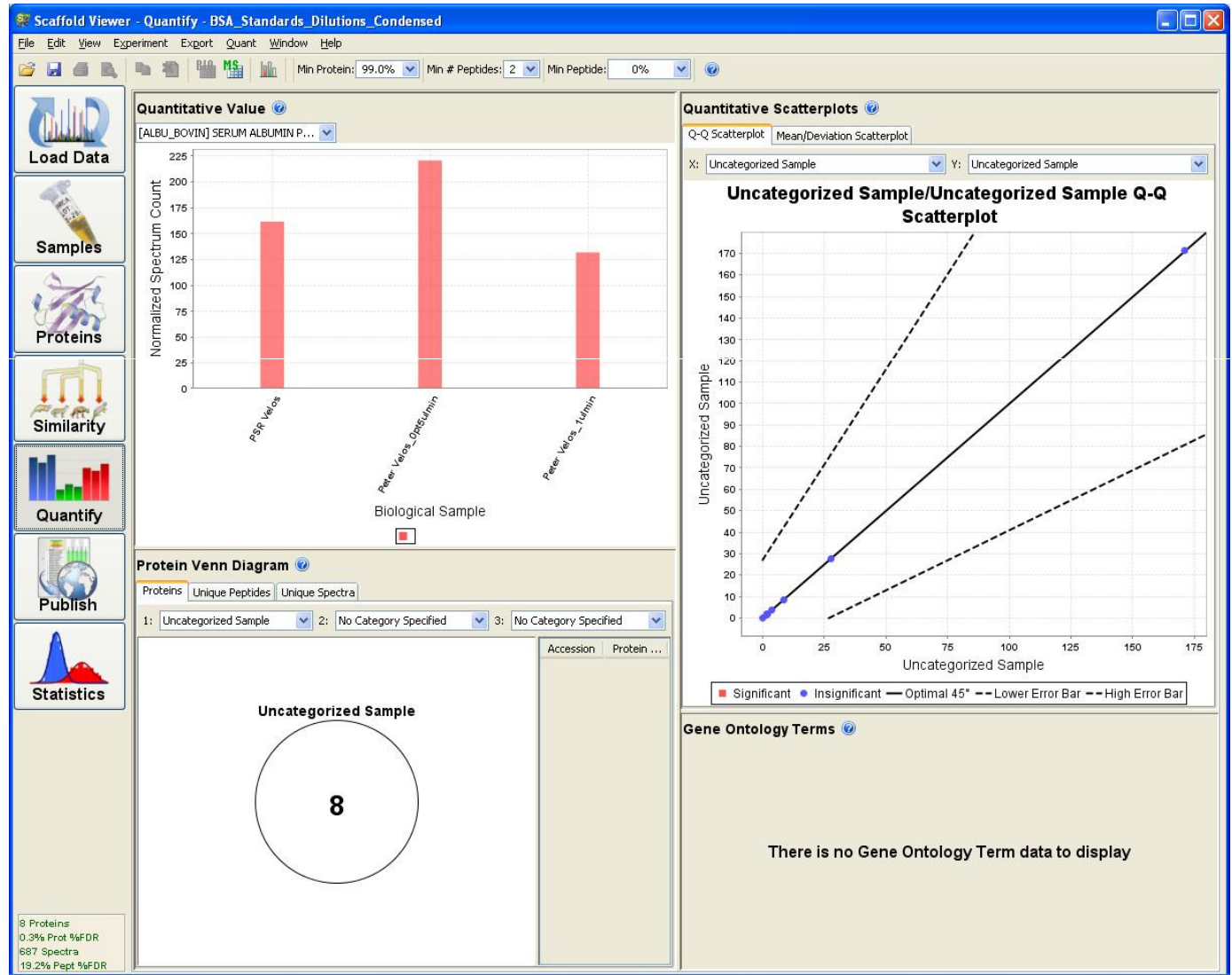
Similarity Tab

- Each peptide is listed along with the proteins it is found in.
- The spectrum viewer at the bottom allows you to critique individual peptide identifications
- Checking or un-checking the “valid” box will add or remove that peptide from your data.
- If all the unique peptides from a protein are removed it will disappear from your list of identified proteins on the Samples tab.



Quantify Tab

- The quantify tab has several options for analyzing your data.
-
- Contact PMAF if you are interested in a more in-depth statistical treatment of your data.



Publish Screen

Click here
To get to
Publish
screen

Then click
here to see
the methods
summary

The screenshot shows a software interface with a sidebar on the left containing icons for 'Load Data', 'Samples', 'Proteins', 'Similarity', 'Quantify', 'Publish', and 'Statistics'. The 'Publish' icon is highlighted. The main window has a menu bar (File, Edit, View, Experiment, Export, Quant, Window, Help) and a toolbar with various icons. Below the toolbar, there are filters for 'Min Protein: 99.0%', 'Min # Peptides: 2', and 'Min Peptide: 95%'. The 'Experiment Methods' tab is active, displaying a table of parameters and values. The 'Publish' button is located at the bottom of the sidebar. The 'Experiment Methods' table lists parameters such as 'Experiment', 'Blank List Generator', 'Version', 'Charge States Calculated', 'Deisotoped', 'Textual Annotation', 'Database Set', 'Database Name', 'Version', 'Taxonomy', 'Number of Proteins', 'Does database contain common...', 'Search Engine Set', 'Search Engine', 'Version', 'Samples', 'Fragment Tolerance', 'Parent Tolerance', 'Fixed Modifications', 'Variable Modifications', 'Database', 'Digestion Enzyme', 'Max Missed Cleavages', 'Scaffold Version', 'Peptide Thresholds', and 'Protein Thresholds'. The 'Publish' button is located at the bottom of the sidebar. The 'Experiment Methods' table lists parameters and values. The 'Publish' button is located at the bottom of the sidebar. The 'Experiment Methods' table lists parameters and values.

Parameter	Value
Experiment:	LO933_20110311_VE_Condensed
Blank List Generator:	
Version:	
Charge States Calculated:	
Deisotoped:	
Textual Annotation:	
Database Set:	1 Database
Database Name:	C:\xcalibur\database\uniprot\uniprot_2010.12\LO933_...
Version:	
Taxonomy:	All Entries
Number of Proteins:	52782
Does database contain common...:	
Search Engine Set:	1 Search Engine
Search Engine:	Sequest
Version:	27, rev. 12
Samples:	All Samples
Fragment Tolerance:	1.00 Da (Average)
Parent Tolerance:	2.0 Da (Average)
Fixed Modifications:	+57 on C (Carbamidomethyl)
Variable Modifications:	+16 on M (Oxidation)
Database:	C:\xcalibur\database\uniprot\uniprot_2010.12\LO933_...
Digestion Enzyme:	Trypsin
Max Missed Cleavages:	2
Scaffold Version:	Scaffold_3_00_08
Peptide Thresholds:	95.0% minimum
Protein Thresholds:	99.0% minimum and 2 peptides minimum

136 Proteins
0.1% Prot %FDR
6538 Spectra
0.3% Pept %FDR

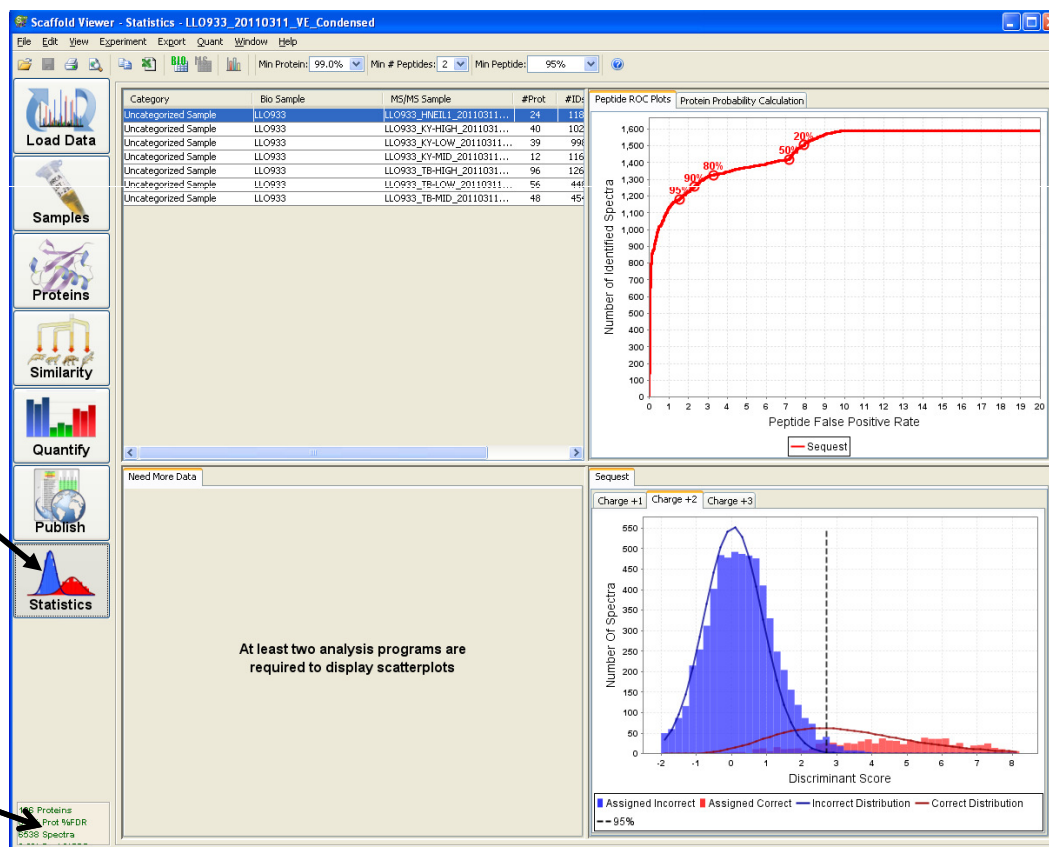
Export Protein Supplemental Material
Export Peptide Supplemental Material

Experimental Methods

- The Experimental Methods tab contains a couple of short paragraphs suitable for the methods section of a paper using the settings that software was run with, the way data has been filtered in the “samples” tab, and variables entered into the lines on the left side of the screen.
- The paragraphs are written on the right side of the screen. This data can be transferred to a document program (i.e. MS Word) by highlighting it, right-clicking and selecting “copy.” Then you can “paste” the paragraphs into the document program with the same method.
- The corresponding data in the “samples” tab can be exported to excel using the tabs on the bottom of the screen
- While helpful the information in this screen is rather generic, and it’s a good idea to contact PMAF and request a methods summary if you are going to present/publish the results.

Statistics

-Displays information relating to the software used to match the MS/MS spectra to the amino-acid sequences in the database, and which make probability estimates based on this information. Note: this page can take a long time to open with larger datasets.

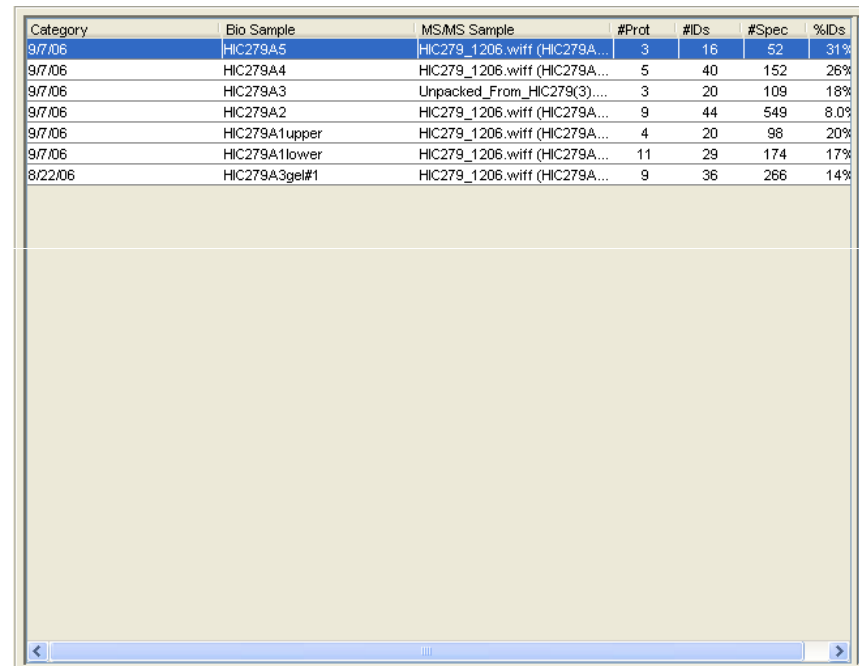


Click here to
View Statistics

There is also a
brief stats
summary
here that can
be viewed
from any page

Upper Left Window

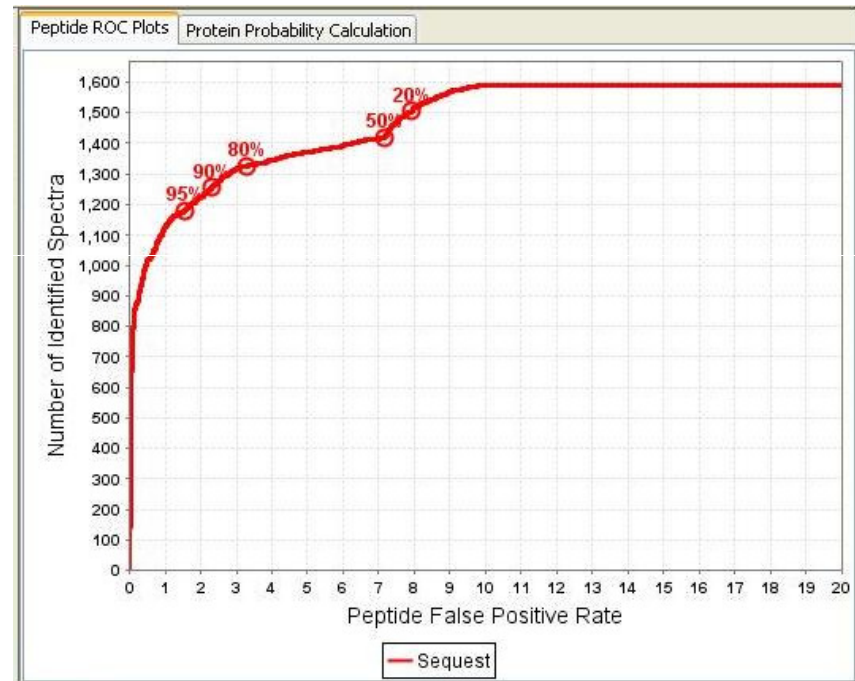
- This window lists the different samples from the “samples” tab
- The data displayed in the other 3 windows is for the sample highlighted in this window
- Note that any of the other fields may be blank if there is not enough data in the sample



Category	Bio Sample	MSMS Sample	#Prot	#IDs	#Spec	%IDs
9/7/06	HIC279A5	HIC279_1206.wiff (HIC279A...	3	16	52	31%
9/7/06	HIC279A4	HIC279_1206.wiff (HIC279A...	5	40	152	26%
9/7/06	HIC279A3	Unpacked_From_HIC279(3)...	3	20	109	18%
9/7/06	HIC279A2	HIC279_1206.wiff (HIC279A...	9	44	549	8.0%
9/7/06	HIC279A1upper	HIC279_1206.wiff (HIC279A...	4	20	98	20%
9/7/06	HIC279A1lower	HIC279_1206.wiff (HIC279A...	11	29	174	17%
8/22/06	HIC279A3gel#1	HIC279_1206.wiff (HIC279A...	9	36	266	14%

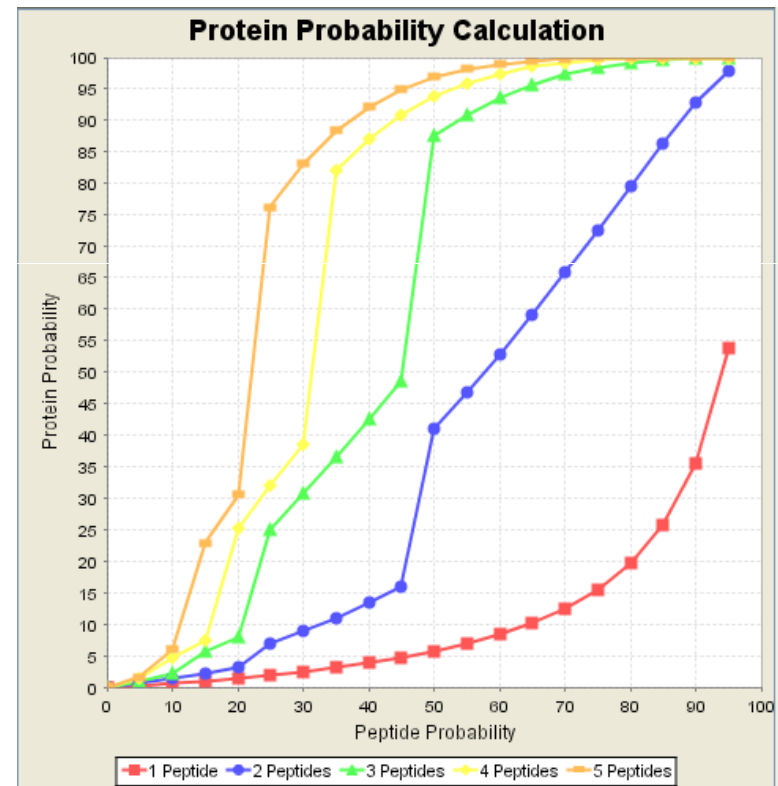
Upper Right Window

- This window displays both a ROC plot and an in-depth analysis of the protein probability calculation.
- The ROC plot displays an estimated peptide FDR against the number of identified spectra.
- Please note that this often greatly underestimates the amount of error, when compared to PSR's internal standards.
-



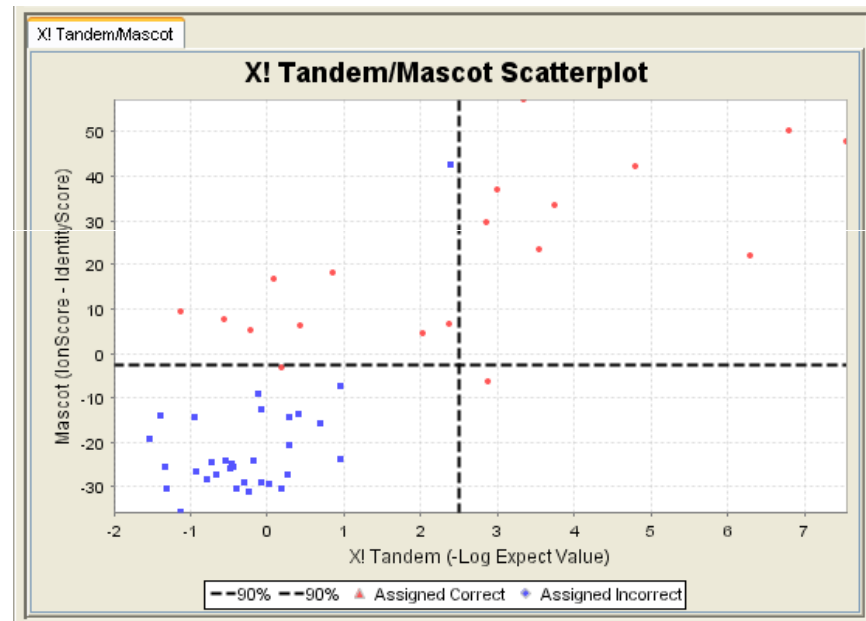
Upper Right Window

- The protein probability window displays the relationship between peptide probability, # of peptides and protein probability
- Note that the # of peptides found strongly affects the protein probability
- Also note that with 95% probability on a single peptide this only relates to about a 50% probability of the protein being present
- Often 2 to 3 high probability peptides are necessary to have a confident protein identification



Lower Left Window

- This window displays a scatter plot of the X! Tandem and other search engine scores for each identified peptide.
- This field is useful for comparing the search engines and evaluating how useful they are to your dataset
- Note that if X! Tandem was not run on your dataset then this field will be blank



Lower Right Window

- This displays the calculated curves which the peptide identification algorithm uses to calculate probabilities
- Scores are sorted by value and 2 curves are matched to the distributions
- The degree of overlap of the two curves relates to the peptide probability

