

## Scaffold Quick Manual

Scaffold files (Proteome Software) can be used for the visualization of the protein and peptide identification results. A free scaffold viewer can be downloaded from:

<http://www.proteomesoftware.com/products/free-viewer/>

Please find below a brief summary of the most relevant Scaffold features. Refer to the official Scaffold manual for detailed information or use the Scaffold Help function.

### Views

#### Samples view



- Define protein & peptide thresholds
- Change display options
- Search for proteins
- Link to protein databases
- Adjustment of taxonomy preferences

Once opened the file, you should set the filter so that only the confidently identified proteins are shown. Please set the filters (at the top) to:

Protein Threshold: 1% FDR (#1)

Min # of peptides: 2 (#2)

Peptide Threshold: 0.1% FDR (#3)

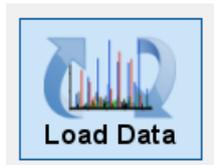
Display options: Total Unique Peptide or Spectra Count (#4)

Once the Scaffold file is opened, you may change these settings to view more protein identifications.

The Scaffold search box allows the user to type in search terms to identify specific proteins by protein names or accession numbers, but it can also filter on peptide sequences and/or spectra information. The Search field accepts regular expressions and filters the results based on accession number or protein name.

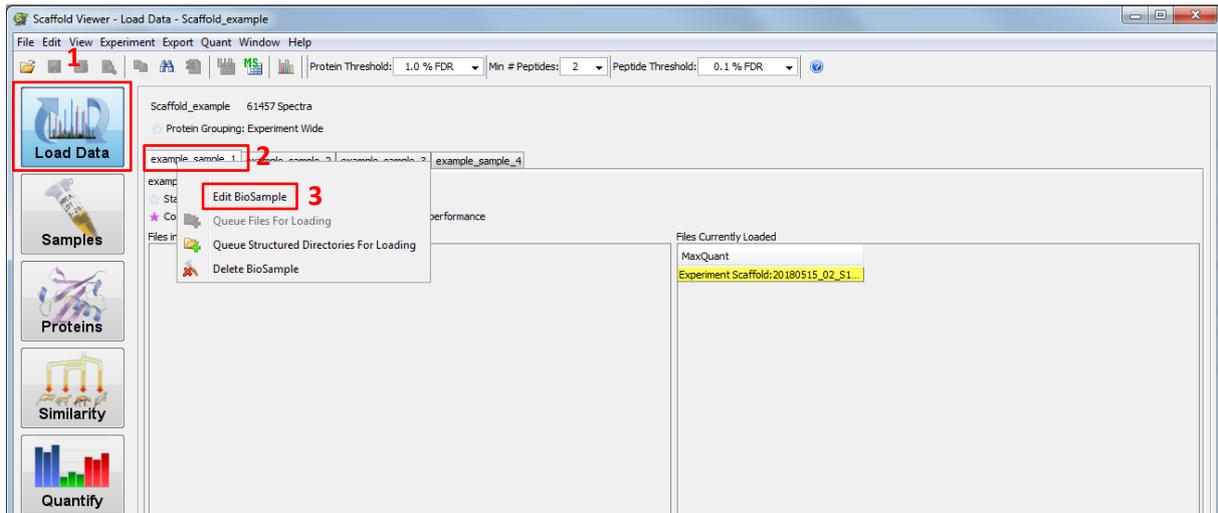
The Advanced Protein Filter dialog allows to filter for, e.g., Protein Sequence Motifs (N.[ST], ...), or presence/absence from 1 or more categories.

## Load Data View

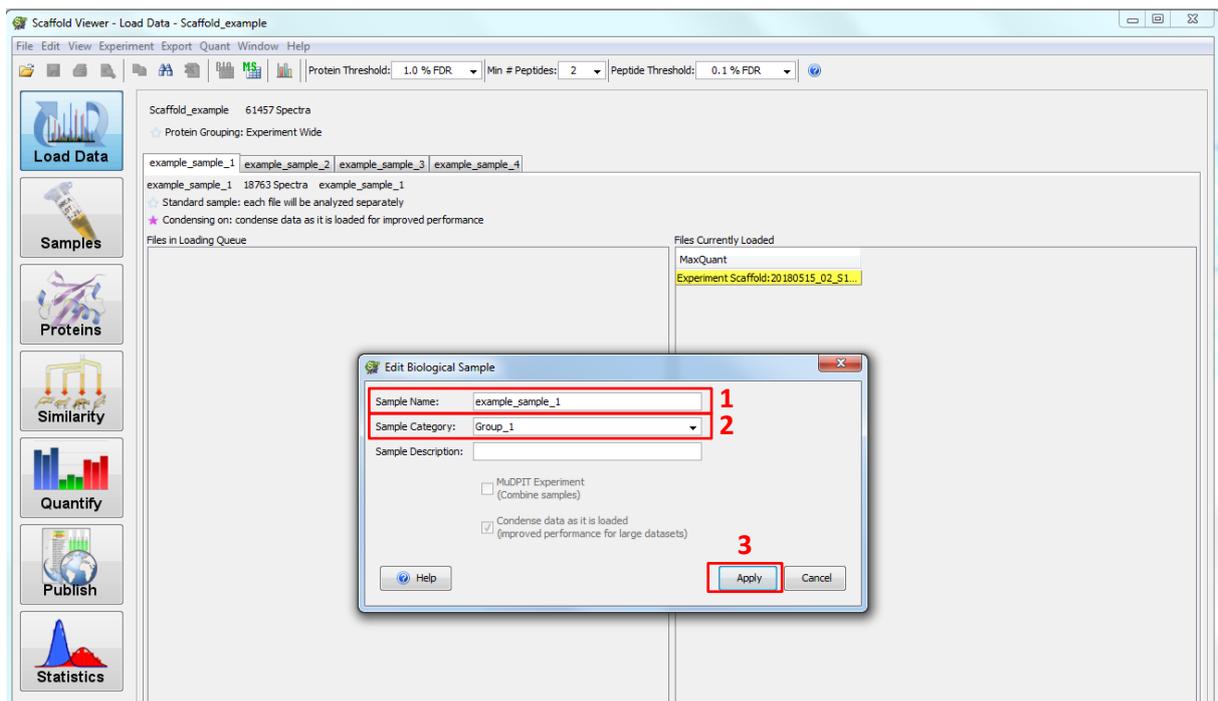


- Rename samples
- Group samples into categories
- Delete samples

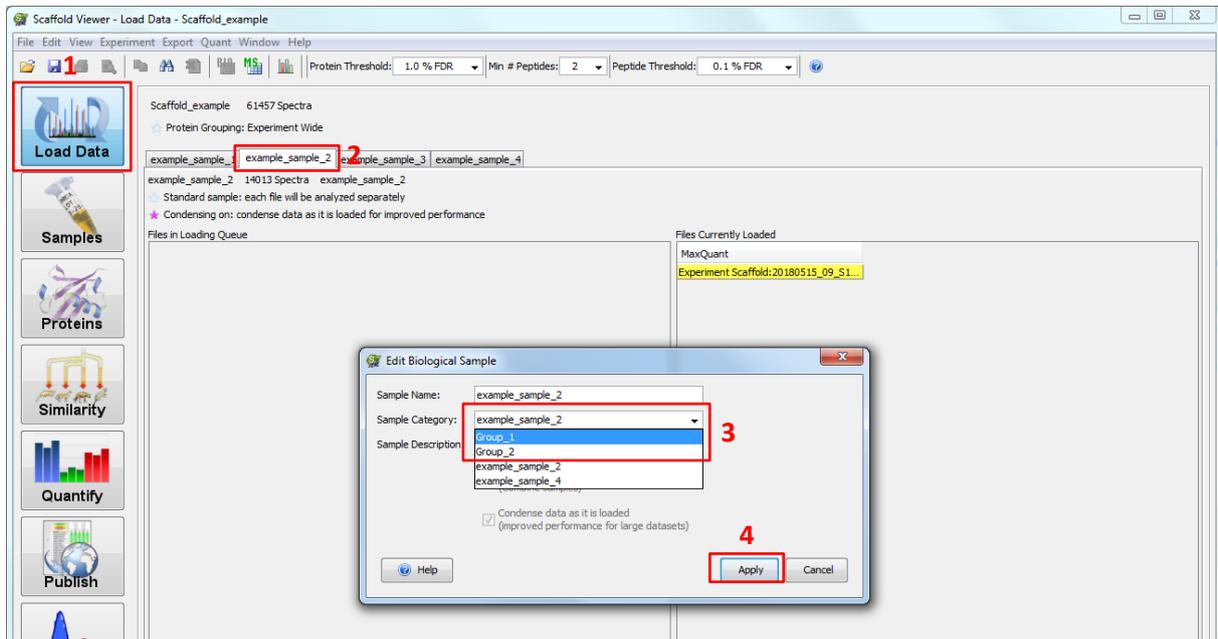
To group the samples go to “Load Data” (#1), do a right mouse click on the sample that you want to change (#2) and select “Edit BioSample” (#3).



In the “Edit Biological Sample” window, the “Sample Name” (#1) and the “Sample Category” (#2) can be changed. To assign a sample to a certain group change the name of the “Sample Category” to your preferred group description (free text). With “Apply” (#3) the changes are saved.



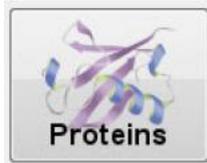
After saving the view changes automatically back to the “Samples” table, change back to “Load Data” (#1) to edit the other samples. Choose the next sample to edit (#2) and with a right mouse click, the “Edit Biological Sample” window opens again. If the description of the group was added already for another sample, the description can now be chosen from the drop-down menu of the “Sample Category” (#3). With “Apply” (#4) the changes are saved.



After all samples are assigned to their group, in the “Samples” (#1) tab you can see which samples belong to which group (#2).

		Probability Legend:					
		over 95 %	80 % to 94 %				
		50 % to 79 %	20 % to 49 %				
		0 % to 19 %					
		Bio View:					
		2155 Proteins in 2060 Clusters					
#	label?	Accession Number	Molecular Weight	Group_1	Group_2	Group_1	Group_2
1	ATP synthase subunit alpha, mitochondrial OS=Mus musculu...	ATPA_MOU...	60 kDa	29	32	27	29
2	ATP synthase subunit beta, mitochondrial OS=Mus musculu...	ATPB_MOU...	56 kDa	28	29	28	28
3	Cluster of Spectrin alpha chain, non-erythrocytic 1 OS=Mus ...	sp P16546...	285 kDa	112	105	94	100
3.1	Spectrin alpha chain, non-erythrocytic 1 OS=Mus musculu...	sp P16546...	285 kDa	111	104	93	99
3.2	Isoform 2 of Spectrin alpha chain, non-erythrocytic 1 OS=...	sp P16546...	282 kDa	110	103	93	98

## Proteins View



- Explore supporting identification data for a specific protein.
- Sequence coverage
- Mass spectra

In the “Proteins” (#1) tab, you get an overview about the sequence coverage, number of peptides, sequence of peptides and the protein sequence. The protein, which is selected in the “Samples” tab, and the proteins from the according cluster are shown.

Scaffold Viewer - Proteins - Scaffold\_example

File Edit View Experiment Export Quant Window Help

Protein Threshold: 1.0 % FDR Min # Peptides: 2 Peptide Threshold: 0.1 % FDR

Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4 All Biological Samples

Valid	Sequence	Prob	P-score	NTT	Modifications	Observed
✓	1.0 (K)NFNINPLAQADWAAK(V)	100%	113.40	2		836.93
✓	1.0 (K)NFNINPLAQADWAAK(K)	99%	35.09	2		836.93
✓	1.0 (K)NFNINPLAQADWAAK(Q)	99%	32.70	2		836.93
✓	1.0 (K)NGFEPASLKE	100%	77.66	2		481.75
✓	1.0 (K)EEVGEAAVELVENGK(K)	99%	28.63	2		872.44
✓	1.0 (K)EEVGEAAVELVENGK(V)	99%	19.30	2		581.96
✓	1.0 (K)EEVGEAAVELVENGK(Y)	100%	115.80	2		624.66
✓	1.0 (K)EEVGEAAVELVENGK(S)	100%	44.04	2	Deamidated (+1)	624.99
✓	1.0 (R)HEMPHIVAITDTAYR(S)	100%	67.42	2		638.98
✓	1.0 (R)HEMPHIVAITDTAYR(S)	100%	63.68	2		638.98
✓	1.0 (R)HEMPHIVAITDTAYR(S)	100%	56.51	2		638.98
✓	1.0 (R)HEMPHIVAITDTAYR(S)	100%	55.01	2		638.98
✓	1.0 (R)HEMPHIVAITDTAYR(S)	100%	38.81	2		638.98
✓	0.5 (K)IQVLHVAASHK(S)	100%	79.89	2		484.94
✓	1.0 (R)TFHFYLLSGAGEHLK(T)	100%	30.42	2		863.98
✓	1.0 (R)TFHFYLLSGAGEHLK(T)	100%	66.83	2		499.76
✓	1.0 (R)TFHFYLLSGAGEHLK(T)	100%	59.35	2		499.76
✓	1.0 (R)TFHFYLLSGAGEHLK(T)	99%	24.11	2		666.02
✓	1.0 (R)TFHFYLLSGAGEHLK(T)	99%	16.31	2		666.02
✓	1.0 (K)TDLLLEPYNK(Y)	100%	109.79	2		603.32
✓	1.0 (R)FLSNHVTIPGQDQK(D)	99%	26.77	2		820.92
✓	1.0 (R)FLSNHVTIPGQDQK(D)	99%	22.99	2		547.62
✓	1.0 (K)DMFQETMEAMR(I)	100%	83.18	2		694.79
✓	1.0 (K)DMFQETMEAMR(I)	100%	49.45	2	Oxidation (+16)	702.78
✓	1.0 (K)DMFQETMEAMR(I)	100%	43.68	2		694.79

Protein Sequence Similar Proteins Spectrum Spectrum/Model Error Fragmentation Table

**MYH9\_MOUSE (100 %), 226378.0 Da**  
**Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4**  
**66 exclusive unique peptides, 92 exclusive unique spectra, 150 total spectra, 872/1960 amino acids (44 % coverage)**

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MAQQAAADKYL YVDK NFINNP LAQADWAAK L VWVPSSKNG FEPASLKEEV GEEAIVELVE
NGKKVKNKDK DIQKMNPPKF SKVEDMAELT CLNEASVVLHN LKERYYSGLI YTYSGLFCVV
INPYKNLPIY SEELVEMYKG KKRHEMPPHI YAITDTAYRS MMQDREDQSI LCTGSEAGAK
TENTKKVIQY LAHVASSHK KKDQGLERQ LLQANPILEA FGNAKTVKND NSSRFGKFI R
INFDVNGYIV GANIEYTLLE KSRAIRQAKE ERTFHFYFYL LSGAGEHLKT DLLLEPYNKY
RFLSNGHVTI PGQDQKDMFQ ETMEAMRIMG IPEDEQMGLL RVISGVLQLG NIAFKKERNT
DQASMPDNATA AQKVSHLLGI NVTDFTRGIL TPRIKVGGRDY VQKAQTKQEA DFAIEALAKA
TYERMFRWLV LRINKALDKT KRQGASFIGI LDIAGFEIFD LNSFEQLCIN YTNEKLLQLF
NHTMFI LEQE EYQR EGI EWN FIDFGLDLQP CIDLIEKPAQ PPGILALLDE ECWFPKATDK
SFVEKVVVQEQ GTHPKFQKPK QLKDKADFCI IHYAGKVVDYK ADEWLMK NMD PLNDNIATLL
HQSSDKFVSE LWKDVDR IIG LDQVAGMSET ALPGAFKTRK GMFRTVGGQLY KEQLAKLMAT
LRNTNPNFVR CIIPNHEKKA GKLDPHLVLD QLR CNQVLEGI IRICRQGFPN RVVVFQEFRQR
YEILTPNSIP KGFMDGKQAC VLMIKALELD SNLYRIGQSK VFFRAGVLAH LEEERDLKIT
DVIIGFQACC RGYLARKAFA KRQOQLTAMK VLQRNCAAYL RLRNWNQWRL FTKVKPLLNS
IRHEDELLAK EAELTKVREK HLA AENRLTE METMQSQLMA EKLQLQEQQLQ AETELCAEAE
ELRARLTAKK QLEEEI CHDL EARVEEEER COYLQAEKKK MQQNIQELLEE QLEEEESARQ
KLQLEKVTTE AKLKKLEEDQ IIMEDQNCCKL AKEKKLLEDR VAEFTTNLME EEEKSKSLAK
LKNKHEAMIT DLEERLREE KQRQLEKTR RKLEGDSTDL SDQIAELMAQ IAE LKMLAK
KEEELQAALA RVEEEAAQKN MALKKIRELE TQISELQEDL ESERASRNKA EKQKRD LGE
  
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2155 Proteins at 81.0 % Minimum  
 2 Min # Peptides  
 0.0 % Decoy FDR  
 59600 Spectra at 11.0 % Minimum  
 0.00 % Decoy FDR

## Quantify view



- Venn Diagrams pane
- Gene Ontology Terms pane

In the “Quantify” (#1) tab, different Venn Diagrams can be shown. One can choose between displaying individual proteins or protein clusters (#2). Once the samples are grouped, one can only compare groups to each other (#3). If single samples need to be compared it either has to be done before grouping or the grouping has to be deleted. It’s possible to compare proteins, total unique peptides or total unique spectra in the Venn diagrams (#4). The panel next to the Venn diagram (#5) shows all proteins (with the given filter criteria). The number of normalized total spectra of the selected protein in #5 is shown in the «Quantitative Value» (#6) view. The values are given for all samples (groups are indicated in same colours).

The Gene Ontology Terms pane gets populated only when GO terms have been searched and found.