Peptide Mapping

Biologics Explorer Software 5.0 Guidelines

Powered by Genedata Expressionist®



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 - Data Review
 - Export and Report Results

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 - 2.Extended Characterization
 - 3.Sequence Variant Search
 - 4.Comparative Analysis
 - 5.Batch Processing
 - 6.Review Snapshots

Part A General Guidelines for Peptide Mapping Workflows



C. D'Britson . BN

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Overview of Applications for Peptide Mapping Workflows



- Use the Peptide Mapping workflows for the analysis of enzymatically digested biotherapeutic molecules, for:
 - Sequence coverage and confirmation
 - Glycopeptide analysis
 - Post-translational modification (PTM) analysis
 - Target PTM profiling

- Disulfide-bond (DSB) analysis
- Conjugate analysis
- Sequence variant analysis (SVA)

- Use Pepmap workflows _Simple, _Extended, _Comparative or _SVA for replicate analyses with common peak boundaries across all samples.
- Use Pepmap workflow _BatchProcessing for analysis of the same or different molecules, with each sample treated as an individual replicate with no shared peak boundaries.
- Use Pepmap workflow _ReviewSnapshots to open saved results from other Pepmap workflows.

Peptide Mapping Workflows



- Pepmap_Simple:
 - A Peptide Mapping workflow for routine characterization, with identification and quantification of the most common modifications and glycosylations.
- Pepmap_Extended:
 - A Peptide Mapping workflow that has more search nodes, for identification of less common PTMs to maximize sequence coverage.
- Pepmap_BatchProcessing:
 - A version of the Pepmap_Extended workflow that analyzes each data file on a sample-by-sample basis.

Pepmap_Comparative:

- A version of the Pepmap_Extended workflow that has activity nodes to complete a differential analysis between sample sets.
- Pepmap_SVA:
 - A version of the Pepmap_Extended workflow that has more search nodes for identification of potential sequence variants.

Pepmap_ReviewSnapshots:

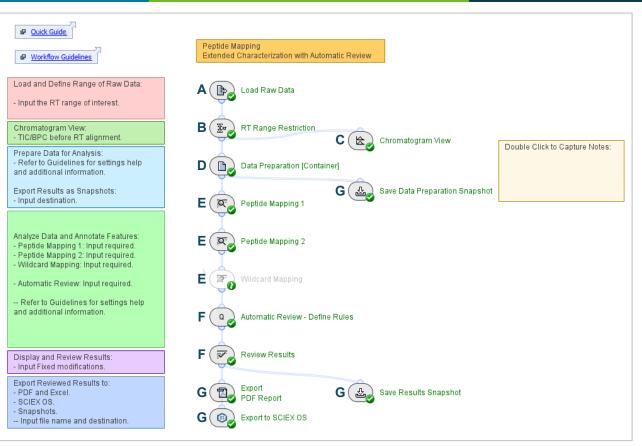
A workflow to open or review saved results.

2. Common Activity Nodes for Peptide Mapping Workflows

GENERAL GUIDELINES FOR PEPTIDE MAPPING WORKFLOWS

Example of a Typical Peptide Mapping Workflow





Common Activity Nodes in Peptide Mapping Workflows

- A. Load Raw Data \rightarrow Input required*
- **B.** RT Range Restriction \rightarrow Input required^{*}
- C. Chromatogram View (before RT alignment)
- **D.** Data Preparation [Container]
 - i. Chromatogram Chemical Noise Subtraction
 - ii. Chromatogram RT Alignment
 - iii. *Chromatogram View* after Alignment
 - iv. Chromatogram Peak Detection
 - Chromatogram Isotope Clustering ν.
 - vi. Singleton Filter
- E. Peptide Identification \rightarrow Input required*
- **F.** Review Results \rightarrow Input required*
- G. Report and Export Results



*Optimize settings for new samples



- vi. Charge Grouping
- vii. Adduct Grouping
- viii. MS/MS Consolidation
- ix. MS/MS Peak Detection
- MS/MS Deisotoping Χ.

Note: For information about Batch Processing, refer to **B:** 5. Guidelines for Peptide Mapping Batch Processing Workflows.

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Load Raw Data: Data Files

- To upload raw data files, click the folder icon 📴.
 - Select container files with the format wiff or wiff2.
 - If data was acquired with the ZenoTOF 7600 system, then select only the wiff2 format.
 - Do not select the auxiliary files with the same name.

□ 20210203 Adalimumab tryptic 2ug ECD_1.timeseries.data X
 □ 20210203 Adalimumab tryptic 2ug ECD_1.wiff.scan X
 □ 20210203 Adalimumab tryptic 2ug ECD_1.wiff2 √

- To select samples in a wiff or wiff2 container file:
 - Double-click the wiff or wiff2 container to see the sample files.
 - Select the samples to upload.

📵 Load Raw Da	ata - Settings	×
General Advan	ced Display	
Name:	Peptide Mapping Extended	1
Format:	SCIEX WiffTwo (*.wiff2) \lor	
	Use File Name as Sample Name	
	Enable Numbering of Samples	
	Enable Raw Data Parsing	
Files/Folders:	20210203 Adalimumab tryptic 2ug ECD_4.wiff2 20210203 Adalimumab tryptic 2ug ECD_5.wiff2 20210203 Adalimumab tryptic 2ug ECD_6.wiff2]
	3 items	
0 🛅	OK Cancel Apply	



Load Raw Data: Use File Name as Sample Name



- The File Name of the wiff or wiff2 container file might not be the same as Sample Name in the wiff or wiff2 container file.
 - The name of the **Data File** in the SCIEX OS software becomes the name of the wiff or wiff2 container file (**File Name**) in Biologics Explorer software.

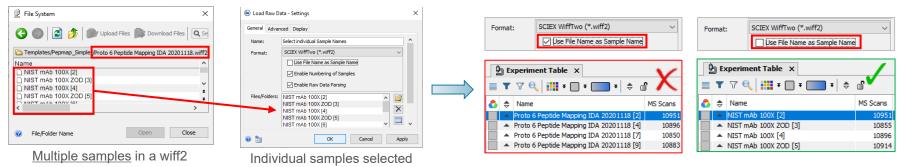
Sample Name MS Method LC Method Rack Typ	e Rack Position Plate Type Plate Position Vial Position Injection Volume (ul) Sample Type Data File Processing Method Results File
Sample Name	File Name
🔮 File System X	 Individual acquisitions with different wiff or wiff2 File Names might
🚱 🌑 💈 🏂 🇊 Upload Files 📦 Download Files 🔍 Se	have the <u>same</u> Sample Name.
	Note: If entries in the Experiment Table have the same Sample Name,
Shared/Adalimumab/20210203 Adalimumab tryptic 2ug ECD_4.wiff2 Name	there can be an effect on the quantitative information reported.
20210203 Adalimumab ECD	Soloot Use File Name as Sample Name in Load Paw Data to use
< >>	• Select Use File Name as Sample Name in Load Raw Data to use
	the File Name in the Experiment Table.
File/Folder Name Open Close	- If Format: Auto Detect is selected, then the Sample Name is used in the
	Experiment Table.

Load Raw Data: Use File Name as Sample Name

To load replicates that have <u>one sample</u> in each wiff or wiff2 container, select Use File Name as Sample Name:



2. To load <u>multiple samples</u> from in the same wiff or wiff2 container, <u>do not</u> select **Use File Name as Sample Name**:



The Power of Precision

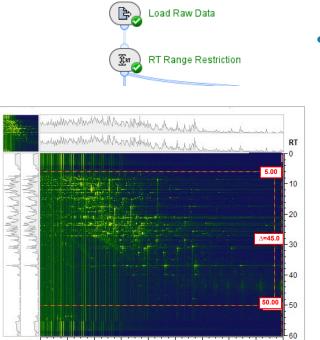
1400 1600

1800

1200

Restrict the RT Range





- To identify the retention time (RT) ranges that contain meaningful data, open (double-click) the results of *Load Raw Data*.
 - Exclude stray signals caused by valve switching or column wash.
 - Focus on the separation range.

(B) RT Range Restriction - Settings			
General Display			
RT Minimum: 5 Minutes RT Maximum: 50 Minutes			
🛞 🛅 OK Cancel Apply			

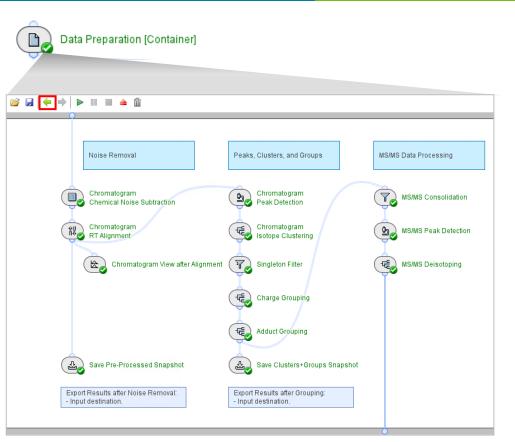
Note: If the fields are blank, or if *RT Range Restriction* has the **Bypass** icon activated, then the full RT range is used.

m/z

400

600 800 1000

Data Preparation [Container]



- Activity nodes in the *Data Preparation* [*Container*] process the raw data for analysis.
 - Only optimize the settings of these activity nodes under specific conditions, for example, when troubleshooting a low sequence coverage.



Chromatogram Chemical Noise Subtraction: Smoothing

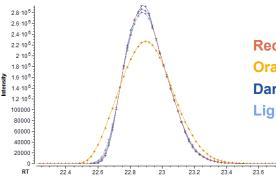




🖲 Chromatogi	am Chemical I	Noise Subtraction - S	Settings X
General Advar	iced Display		
- 🔽 Chromat	ogram Smoothin	g	
RT Window:	5	Scans	
Estimator:	Moving Averag	e	\sim
Chemica	l Noise Subtracti	on ———	
RT Window:	201	Scans	
Quantile:	60 %		
Method:	Clipping) Subtraction	
Threshold:	14	[Intensity]	
		_	
0 🛅	OK	Cancel	Apply

Chromatogram Smoothing is used to improve the RT profile of peaks for peak detection.

- Estimator:
 - **Moving Average** replaces the intensity of each data point with the mean average intensity of the data points in the **RT Window**. High values cause peak widths to increase, but peak volume is not changed.
 - **Binomial** is an iterative form of **Moving Average** that has less effect on peak widths at high scan values.



Red: Moving Average (5 scans) Orange: Moving Average (15 scans) Dark Blue: Binomial (5 scans) Light Blue: Binomial (15 scans)

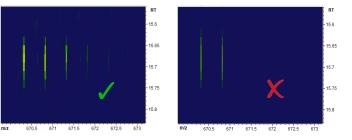
Chromatogram Chemical Noise Subtraction





neral Advar	nced Display
Chromat	togram Smoothing
RT Window:	5 Scans
Estimator:	Moving Average \checkmark
	Noise Subtraction
T Window:	201 Scans
T Window: Quantile:	201 Scans 60 %
Chemica T Window: Quantile: Method:	201 Scans

- Chemical Noise Subtraction decreases the length of long-tailing peaks.
- Change this setting if the default values remove too much signal.
- If too much signal if removed, it can be identified by:
 - Excessive cutoff of the tails of very wide (extended RT) peaks.
 - Loss of low-intensity isotope peaks from singly (+1) or doubly (+2) charged clusters, or from low-intensity clusters of interest:



- To decrease the amount of noise removal (keep more signal):
 - Decrease the **Quantile**.
 - Increase the RT Window.

Chromatogram Chemical Noise Subtraction: RT Window

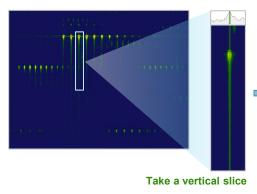


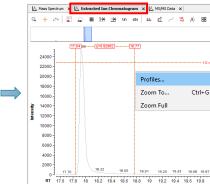
~			
Chromatog	ram Chemical	Noise Subtraction - Settin	gs X
General Advar	nced Display		
Chroma	togram Smoothir		
RT Window:	5	Scans	
Estimator:	Moving Averag	je	\sim
	al Noise Subtract	ion	
RT Window:	201	Scans	
IXT WINGOW	201	beans	
Quantile:	60 %	Scans	
Quantile:	60 %		
Quantile: Method:	60 %) Subtraction	
Quantile: Method:	60 %) Subtraction	

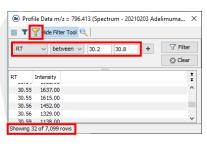
• Generally, the **RT Window** should be at least double the number of scans across the largest peak in the dataset.

To calculate the number of scans:

- 1. Find a feature that extends over a longer RT than other features in the ion map.
- 2. Take a vertical slice to create an Extracted Ion Chromatogram.
- 3. Right-click in the Extracted Ion Chromatogram window, and then select Profiles.
- 4. Use the **Advanced Filter Tool T** to select the RT range for the peak.
- 5. Record the number of scans shown, and then enter at least double this value for the **RT Window** in the settings.







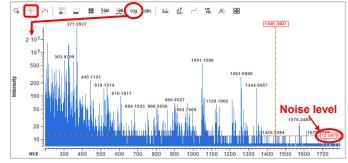
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Chromatogram Chemical Noise Subtraction: Threshold



Chromatogi General Advar	j-	×
	togram Smoothing	
RT Window:	5 Scans	
Estimator:	Moving Average \checkmark	
Chemica	Noise Subtraction	
RT Window:	201 Scans	
Quantile:	60 %	
Method:	Clipping Subtraction	
Threshold:	14 [Intensity]	
0 🛅	OK Cancel Apply	

- If the noise level is significantly different from the Threshold value pre-set in the Chromatogram Chemical Noise Subtraction activity node, then change this setting.
- To measure the noise level and identify an applicable **Threshold** intensity value:
 - 1. Drag the intensity axis of the mass spectrum until the noise level can be seen, or use the icon in the tool bar to change the axis from the linear to the logarithmic scale.
 - 2. Use the crosshair tool + to measure the intensity of the noise level.

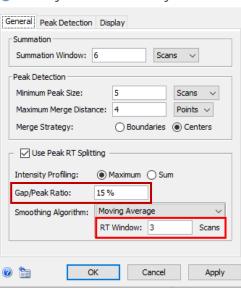


A: 2.GENERAL GUIDELINES FOR PEPTIDE MAPPING WORKFLOWS

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Chromatogram Peak Detection: Use Peak RT Splitting

- Shoulder peaks, in the RT direction, can be detected as a single peak, or as multiple separate peaks.
 - To increase the number of peaks detected (increase split sensitivity):
 - Decrease the Gap/Peak Ratio.
 - Decrease or remove **Smoothing**.
 - Use Peak RT Splitting Maximum
 Sum Intensity Profiling: 15 % Gap/Peak Ratio: 2310 19 Moving Average Smoothing Algorithm: RT Window: 5 Scans 10.8 10.9 11 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 17.1 17.2 17.3 17.4 17.5 17.6 17.7 17.8 16.9 17 Gap/Peak Ratio: 30 % Smoothing Algorithm: None Higher split sensitivity Lower split sensitivity More peaks Fewer peaks 17.1 17.2 17.3 17.4 17.5 17.6 17.7 17.8 10.8 10.9 11 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 16.9 17 © 2024 DH Tech, Dev. Pte. Ltd.





Chromatogram Peak Detection - Settings



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20000

m/z 838

839

840

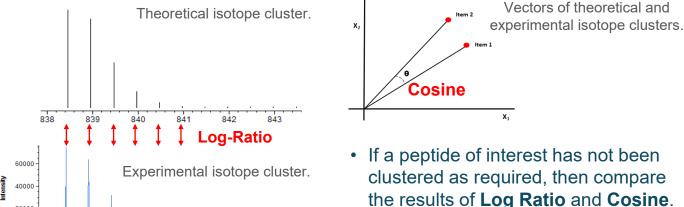
841

842

Chromatogram Isotope Clustering: Distance Measure



- The **Distance Measure** compares each experimental peak to the theoretical isotope profile for that peptide.
- × Chromatogram Isotope Clustering - Settings General Envelope Fitting Advanced Display Method: Peptide Isotope Shaping Ionization: Protonation Minimum Charge: Maximum Charge: 22 Distance Measure: Cosine Max. Distance: 0.6 Recompute Mono-Isotopic Peak Mass Threshold: 3000 Da 🕜 🛅 OK Cancel Apply
- Log Ratio treats all peaks in a cluster equally.
- **Cosine** converts each cluster into a vector, with the contribution of each peak relative to the abundance. Therefore, smaller peaks have less impact.



843

The Power of Precision

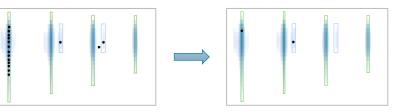
MS/MS Consolidation: Merge Across Chromatograms



- This activity node merges MS/MS data across equivalent peaks and clusters.
 - Consolidation can improve MS/MS spectra, and increase identifications.
 - Consolidation can decrease false positives if MS/MS spectra are too ambiguous.

MS/MS Consolidation - Settings							
General Advanced Display							
Remove:	MS/MS not in Features (Autodetect) \lor						
Observable:	Max. Intensity 🗸 🗸						
Consolidation:	Merge	Merge 🗸 🗸					
	Level:	One per Cluster 🗸 🗸					
		Across Chr	omatograms				
	m/z Tolerance:	0.1	Da				
	Min. Similarity:	0					
Filter MS/MS Peaks							
Max. Number of Peaks: 100							
Min, Peak Inter	nsity: 5						
0	OK	Cancel	Apply				

MS/MS Consolidation



- To use MS/MS data from technical replicates to increase confidence in identifications, select to **Merge** MS/MS data **Across Chromatograms**.
 - Merged MS/MS data are assigned to the sample with the highest intensity for that spectrum.
 - To measure individual sample sequence coverage, <u>do not</u> select this option.

Coverage				
Tra_HC				
	Total	Mass Only	MS/MS Only	Mass and MS/MS
Overall	94.0%	0.0%	0.0%	94.0%
Trastuzumab [1]	94.0%	0.4%	0.0%	93.6%
Trastuzumab [2]	94.0%	0.4%	0.0%	93.6%
Trastuzumab [3]	94.0%	0.4%	0.0%	93.6%
Trastuzumab [4]	94.0%	0.0%	0.0%	94.0%

Across Chromatograms

Coverage				
Tra_HC				
Overall Trastuzumab [1] Trastuzumab [2] Trastuzumab [3] Trastuzumab [4]	Total 94.0% 94.0% 94.0% 94.0% 94.0%	Mass Only 0.0% 34.7% 7.1% 21.3% 10.0%	MS/MS Only 0.0% 0.0% 0.0% 0.0% 0.0%	Mass and MS/MS 94.0% 59.3% 86.9% 72.7% 84.0%
Across Chromato	grams			

MS/MS Consolidation: Extend Peak Boundaries

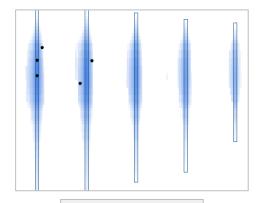




B MS/MS Conso	MS/MS Consolidation - Settings			
General Advance	d Display			
Extend Pea	ak Boundaries			
m/z Tolerance:	0.2	Da 🗸		
RT Tolerance:	0	Minutes		
	0	Minutes		
0	ОК	Cancel	Apply	

To analyze data acquired from a TripleTOF 5600/5600+, TripleTOF 6600/6600+, or X500B QTOF mass spectrometer, activate the Extend Peak Boundaries option.

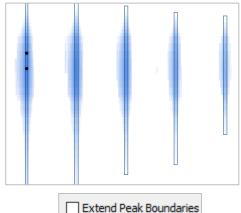
- This setting includes MS/MS data located outside of the peak boundaries in the consolidation.



Extend Peak Boundaries

MS/MS data outside of the

peak boundaries is kept.



Extend F

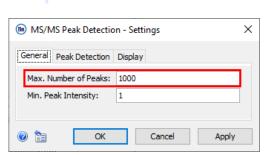
MS/MS data outside of the peak boundaries is removed.

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MS/MS Peak Detection

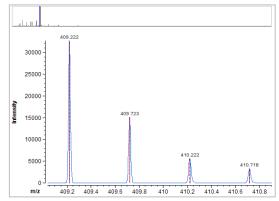


- This activity node detects peak centroids in MS/MS profile data.
 - Peak centroids are required for Peptide Mapping.



MS/MS Peak Detection

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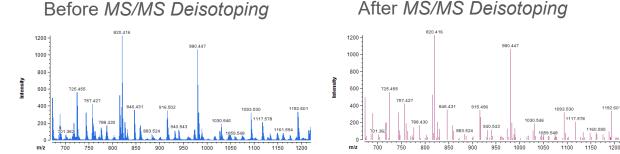
- To increase the likelihood of identifying long (high mass) peptides with EAD data, increase the Max. Number of Peaks to 5000 to keep more of the low-intensity MS/MS ions.
 - The number of MS/MS peaks detected, compared to the number of MS/MS peaks used for identification, has an effect on the score.
 - To compensate for the higher number of peaks, decrease the **Min. Score** in *Peptide Mapping.*
 - Note: A lower score increases the risk of false positive identifications.

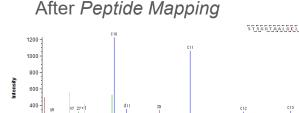
MS/MS Deisotoping



MS/MS Deisotoping

- This activity node removes the isotopic peaks in high-resolution MS/MS data to give singly-charged (deisotoped) monoisotopic peaks.
 - Deisotoping decreases the number of MS/MS peaks in the fragment spectra.
 - The number of MS/MS peaks detected, compared to the number of MS/MS peaks used for identification, has an effect on the score.





200

Peptide Mapping: General

X

Display

Crosslinks

~

Apply

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Peptide Mapping	
-----------------	--

Peptide Chromatograms

EAD

70

Top Ranked

OK

ppm ~

ppm 🗸

Cancel

Peptide Mapping 1 - Settings

Sequence

Mass-only Matches: Discard all

Ignore Annotated Features

Export Coverage Data (deprecated)

MS/MS Identification

Conjugates

Instrument:

Min. Score:

Keep:

🕜 🛅

m/z Tolerance:

Mass Tolerance: 8

General

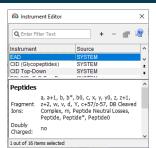
- **Instrument**: Select the fragmentation type used for data acquisition.
 - To review or change the types of fragment ions used for identification:
 - Browse to File > Tools > Instrument Editor.

m/z Tolerance:

- The m/z Tolerance value is not an indicator of the instrument mass accuracy. It adjusts for the possible impact on the m/z of MS/MS pre-processing.
 - Decrease the **m/z Tolerance** to decrease the number of false positive or ambiguous annotations.

Min. Score:

- The number of MS/MS peaks has an effect on the optimal score threshold.
 - The MS/MS score is related to the number of identified and unidentified peaks in the spectrum.
 - Information-rich MS/MS spectra with many peaks can be confidently identified with a lower score.
- Increase the Min. Score to decrease the number of false positives or ambiguous annotations.



Peptide Mapping: Sequence



	- Settings	×
Conjugates General Sequence	Peptide Chromatograms Modifications Glyo	Report Display cosylation Crosslinks
Sequence(s):	From Text	~
	>LC DIQMTQ >Trypsin IQVRLGE >rLysC	GGGLVQPGRSLR
Enzymes:	Trypsin	+
Max. Missed Cleavag	es: 4	Q Enter i

Peptide Mapping

- Sequence(s):
 - If all samples have the same sequence, enter it in one of these formats:
 - From Text: Enter the protein sequence in the Sequences box.
 - From Fasta File: Select a FASTA file that contains the sequence of interest.
 - **From Global File**: Select a FASTA file with multiple entries, and then select the sequences to use from the pop-up window.
 - If different samples require different sequences:
 - Use the Batch Processing workflow.

Note: For more information about Batch Processing, refer to the section **B**: 5.Guidelines for Peptide Mapping Batch Processing Workflows.

Enzymes:

×

Cancel

OK

- To see the list of system-configured and user-defined enzymes, use the + icon to open the Select Entries dialog.
- Adjust enzyme specificity, maximum number of missed cleavages, and minimum peptide length as required.

Peptide Mapping: Modifications



- To review existing modifications or to add custom modifications:
 - Browse to File > Tools > Modification Editor.

☆

☆

Cancel

Modification Editor						×
Q Enter Filter Text			+	- 1	r	0
Modification	Gain	Loss	Ма	ss delta	s	^

Peptide Mapping	1 - Settings	×
Conjugates General Sequence	Peptide Chromatograms Report Display ee Modifications Glycosylation Crosslinks	Select Entries
Fixed:	Carbamidomethyl (C)	C. Enter Filter Text Deamidated (NQ) Gin->pyro-Glu (N-term Q)
Variable:	Deamidated (NQ) Maximum: 2 per Sequence Allowed: Anywhere Unmodified: is Required Max. Distance: 2.0 Minutes Same Charge: true Allow Glyccosylation: true	Lys-loss (Protein C-term K) 2 -dimethylsuccinyl (C) CK
	Gln->pyro-Glu (N-term Q) Maximum: 1 per Sequence Allowed: Anywhere Unmodified: is not Required Allow Glycosylation: true	
Sequence Variants:	No Variants 🗸 👘	• To
Maximum:	3 per Peptide	
		M
	Properties	×
0	Ammonia-loss Deamidated Gln->pyro-Glu Glu->	>pyro-Glu Lys-loss Oxidation
	Maximum: 2 per Seque	
	Allowed: Anywhere	~ 2
	Unmodified: is Required Max. Distance: 2.0 Same Charge:	Minutes
	Allow Glycosylation: 🔽	

Modifications tab:

- To see the list of available **Fixed** or **Variable** modifications, use the **+** icon to open the **Select Entries** dialog.
 - To add commonly used modifications as favorites, select the star ☆ icon.
- To edit the properties of modifications selected for a particular Peptide Mapping search:
 - 1. Select all Variable or Fixed modifications to edit.
 - 2. Use the ropen the **Properties** dialog.
 - Select if the **Unmodified** peptide is required for the modification to be identified, and if so, how closely the two forms must elute.

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Peptide Mapping: Glycosylation (1)



×

Upload Files

S A

🐵 Peptic	de Mapping 1 - Settings	×
Conjug General		Display Crosslinks
Type:	Glycosylated	_ ~
	Library: CHO N-Glycans medium	\sim
	Allowed Sites: Only N-Linked	\sim
	Use Consensus Sequences:	
	Filter for Core Structures:	
	Max. Number of Glycans per Peptide: 1	
	Substituents:	+
		-
	Max. Substitutions: 1	
0 🛅	OK Cancel	Apply

Glycosylation tab:

- **Library**: Select a system-configured or user-defined library.
 - To review or change a glycan library: Browse to File > Tools > Library Browser > Resources.
 - A search for multiple glycosylation sites can produce a high number of results and take a long time to complete. General guidance to control the search space:
 - **Max. Number of Glycans per Peptide**: For the search to proceed, the predefined threshold number of Estimated Glycopeptide Candidates cannot be exceeded.

Note: For more information, refer to the next page: *Peptide Mapping*: Glycosylation Tab (2).

- Allowed Sites: Only *N*-linked:
 - Max. Number of Glycans per Peptide: The maximum allowed value is 4.
 - Fewer missed cleavages and variable modifications decreases the search time.
- Allowed Sites: Only O-linked: The search criteria must be controlled because every serine
 (S) and threonine (T) residue is a potential O-glycosylation site.
 - Long peptides that contain many potential glycosylation sites have a large effect on the number of Estimated Glycopeptide Candidates, and the subsequent processing time.
 - To decrease the total number of candidates and the search time, use enzymes that create shorter peptides. For example, select Trypsin/P so that cleavage is <u>not</u> restricted at RP/KP.

Library Browser

D 📂

File System

Resources

glycomedb.gwd

cho n-glycan large.gwd

) cho_n-glycan_med.gwd) cho_n-glycan_small.gwd

Peptide Mapping: Glycosylation (2)



Example of permitted search combinations for *O*-glycans in Etanercept digested with trypsin:

Enzyme: Trypsin Missed cleavages: 1

Glycans/ peptide

3

4

5

6

7



Chronnel		size of	giycar	librar	/
Glycans/ peptide	3	4	5	6	7
3	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
4	✓	✓	 Image: A start of the start of	✓	✓
5	✓	✓	~	✓	×
6	~	✓	~	×	×
7	×	✓	×	×	×

Enzyme: Trypsin/P

🙆 Edit Instrument	×
General Peptides Glycans Glycopeptides	
Peptide Fragments: 🔽	
Glycan Fragments:	
OK Can	cel

Note: Disable **Glycan Fragments** for glycopeptides in the **Edit Instrument** settings to decrease the time required for complex glycan searches. For glycopeptide analysis of CID data, enable **Glycan Fragments** for better MS/MS coverage.

Glycosylation tab:

- A combination of factors is used to calculate the number of Estimated Glycopeptide Candidates, which controls if the search will proceed:
 - 1. The number of glycans in the glycan library (including substituents).
 - Use the smallest library size that contains the applicable glycans of interest.
 - Select Filter for Core Structures to decrease the number of candidates.

2. The Max. Number of Glycans per Peptide.

- Enter the estimated number of glycans for the molecule. Larger libraries can be used with fewer glycans per peptide, and *vice versa*.
- 3. The theoretical sites of glycosylation on a peptide.
 - The number of missed cleavages and the enzyme specificity have an effect on the total number of theoretical sites of glycosylation.
- Other search parameters have an effect on the overall search time.
 - To decrease the time to completion:
 - Disable Glycan fragments in the Edit Instrument settings.
 - Minimize the variable modifications and their number per peptide.
 - Maximize the minimum peptide length.
 - Decrease the number of glycans per peptide.
 - Decrease the size of the glycan library.

Peptide Mapping: Crosslinks (1)



Conjugates eneral Sequer	Peptide Chromatograms nce Modifications G	Report ycosylation	Display Crosslinks
Connectivity			
Fixed			~
Bonds: Max. Peptides:	Disulfide[HC:22, HC:96] Disulfide[HC:148, HC:204 Disulfide[HC:244, LC:214 Disulfide[HC:230, HC:230 Disulfide[HC:233, HC:233 Disulfide[HC:265, HC:325]]]	< >
_	OK	Cancel	Apply
Peptide Mapping	g 1 - Settings Peptide Chromatograms	Cancel Report ycosylation	Apply Disolav Crosslinks
Peptide Mapping Conjugates eneral Sequer	g 1 - Settings Peptide Chromatograms	Report	Display
Connectivity De Novo	g 1 - Settings Peptide Chromatograms Ice Modifications Gh	Report	Display
Peptide Mapping Conjugates eneral Sequer	g 1 - Settings Peptide Chromatograms	Report ycosylation 25]	Display
Deptide Mapping Conjugates Ineral Sequer Ionnectivity De Novo Max. Peptides:	2 Disulfide [Cys-Cys Loss: H2] Thioether [Cys-Cys Loss: H2]	Report ycosylation 25]	Display

Crosslinks tab:

- For <u>reduced</u> samples: Set **Connectivity** to **None**.
- For <u>non-reduced</u> samples, do one of the following:
 - 1. Set Connectivity to Fixed.
 - Use the correct syntax to enter the known disulfide bonds: enter the type of crosslink, and then the protein and amino acid position for each bond.
 - For example: Disulfide[HC:22, HC:96] or Trisulfide[HC:371, LC:194]
 - The chain names must be identical to those specified in the **Sequence** tab.
 - Different crosslinks cannot contain the same location.
 - Enter the number of crosslinked peptides to include in the search.
 - A Max. Peptides value of 3 or more increases the search time.
 - 2. Set Connectivity to De Novo.
 - Use the + icon to select the crosslinkers of interest.
 - Enter the number of crosslinked peptides to include in the search.
 - A Max. Peptides value of 3 or more increases the search time.
 - Enter the expected bonds in the Crosslinks tab of Automatic Review.
 Note: For more information, refer to the page: Automatic Review: Crosslinks in this section (A: 2. General Guidelines for Peptide Mapping Workflows).



Peptide Mapping: Crosslinks (2)



P	eptide Mapping
Peptide Mappi	oing 1 - Settings
Conjugates General Seq	Peptide Chromatograms Report Disolav uence Modifications Glycosylation Crosslinks
Connectivity	
Max. Peptide	s: 4
Crosslinker:	Disulfide [Cys-Cys Loss: H2] +
2 🛅	OK Cancel Apply

🐵 Peptide Mapping 1 - S	ettings		×
Conjugates Pep General Sequence	tide Chromatogra Modifications	ms Report Glycosylation	Display Crosslinks
Enzymes:	Trypsin		+ ^
Max. Missed Cleavages:	3		
Min. Length:	5		
Max. Peptide Length:			*
0	OK	Cancel	Apply

The **De Novo** search can take a long time to complete and create a large number of possible crosslinked peptides. For the search to start, the number of possible crosslinked candidates cannot be more than the threshold of 2.5 million.

- The following *Peptide Mapping* parameters have an effect on the number of Crosslink Candidates:
 - Crosslinks tab:
 - Max. Peptides: Enter the maximum number of peptides in a complex.
 - A Max. Peptides value of 3 or more increases the search time.
 - Sequence tab:
 - Max. Missed Cleavages: Enter the maximum number of missed cleavages in a peptide.
 - In a crosslinked complex, <u>each individual peptide</u> can have up to this number of missed cleavages.
 - Min. Length: Enter the minimum length of a feature.
 - In a crosslinked complex, the minimum length is the total length of <u>all peptide chains</u>. For example, the crosslinked complex XX=XXXXX has a length of 7.

• Max. Peptide Length: Enter the maximum length of a <u>peptide</u> in a complex.

- In a crosslinked complex, the maximum length is the length of <u>each individual peptide</u>.

Automatic Review: Ambiguous Annotations





Automatic Review - Define Ru	les - Settings		×
Isomer Detection	Crosslin	ks	Display
General Modification RT V	Vindows	MS/MS C	overage
Detect Ambiguous Annotations:			
@ 🗎 🛛 O	к	ancel	Apply

Peptide Mapping 1	- Settings >
	tide Chromatograms Report Display Modifications Glycosylation Crosslinks
Mass Tolerance: 8	ppm ~
MS/MS Identifica	ation
Instrument:	EAD ~
m/z Tolerance:	50 ppm ~
Min. Score:	70
Keep:	By Rank 🗸
	Maximum Rank: 3
Mass-only Matches:	Discard all 🗸 🗸
Ignore Annotated	d Features Data (deprecated)
Export Coverage	

- Use *Automatic Review* to define the criteria to highlight *Peptide Mapping* results that require manual inspection.
 - Information is added to the **Flag** or **Comment** columns for the applicable entries in the **Peptide Table** in *Review Results*.

General tab:

To add Ambiguous annotation to the **Comment** column in the **Peptide Table** for features that have multiple annotations, select **Detect Ambiguous Annotations**.

	Peptide Ta	ble 🗙 📴 Modifications Table 🗙 🔣 Peptide Chromat	ogram Table 🗙								
	🕇 🕹 🕑	Review									
	Range	Peptide	Modifications	Mod. Locations	Calc. Mass	Flags	Comment 1	Group Id	RT	Consolidated Score	Charge States
128	HC[419-443]	SRWQQGNVFSCSVMHEALHNHYTQK	Ammonia-loss, Carbamidomethyl	[N425] [C429]	3026.366		Ambiguous annotation	730	12.777	158.069	4:5
130	HC[419-443]	SRWOOGNVFSCSVMHEALHNHYTOK	Ammonia-loss, Carbamidomethyl	[N438] [C429]	3026.366	I	Ambiguous annotation	730	12.777	130.557	4:5
132	HC[419-443]	SRWQQGNVFSCSVMHEALHNHYTQK	Carbamidomethyl, Deamidated	[C429] [Q423]	3044.377		Ambiguous annotation	179	13.639	91.452	5
133	HC[419-443]	SRWQQGNVFSCSVMHEALHNHYTQK	Carbamidomethyl, Deamidated	[C429] [N438]	3044.377		Ambiguous annotation	179	13.639	110.640	5
134	HC[419-443]	SRWQQGNVFSCSVMHEALHNHYTQK	Carbamidomethyl, Deamidated	[C429] [Q442]	3044.377		Ambiguous annotation	179	13.639	94.177	5

Note: For more information on this example, refer to the page: *Review Results*: Isomer Differentiation in this section (**A**: 2. General Guidelines for Peptide Mapping Workflows).

Automatic Review: Modification RT Windows



Auto	matic Review - De	efine Rules	
Υ -	Select Entries	×	
	Q Enter Filter Text		
	Deamidated	^	
	✓ Oxidation		
	Deamidated (IsoAsp)	×	
Automatic Review -	ОК	Cancel	×
Isomer Detection General Modifi	Crosslink cation RT Windows	s Dis MS/MS Cove	play rage
Oxidation Valid Modification RT W Start: <undefined> End: <undefined> Invalid Modification RT Start: -0.5 Minutes</undefined></undefined>	findow Properties		+ ^ -
End: 0.5 Minutes	Oxidation		
Auto-reject: false	Valid Modification RT Wir	ndow	~
0 🛅	Start: End:	Minutes Minutes	Apply
	Invalid Modification RT V	Nindow	
	Start: -0.5	Minutes	
	End: 0.5	Minutes	
	Auto-reject:		

Modification RT Windows tab:

- To see the list of available modifications, use the + icon to open the Select Entries dialog.
 - 1. Select the modifications to monitor with Automatic Review.
 - Note: Deamidated and Deamidated (IsoAsp) are two separate modification entries in the dialog.
 - 2. Enter a time window when a modified peak must be detected in relation to the unmodified peak.
 - Valid Modification RT Window: Modified peaks with a RT outside of this window have a Q (Questionable) in the Flag column and information added to the Comment column.
 - **Invalid Modification RT Window**: Modified peaks with a RT inside this window have a Q (Questionable) in the **Flag** column and information added to the **Comment** column.

Modifications	Flags	Comment	RT
Carbamidomethyl, Oxidation	Q	Oxidation RT window rule violated (unmodified molecule at RT 12.7770)	12.490
Oxidation	Q	Oxidation RT window rule violated (unmodified molecule at RT 7.7863)	7.652

• To automatically reject peptides that do not meet the specified criteria, select **Auto-reject**.

Automatic Review: MS/MS Coverage





🐵 Automatic Rev	view - Define Rules	- Settings		×	
Isomer De General	tection Modification RT Wir	Crosslinks Idows	Dis MS/MS Cove	solav erage	
Min. Fragment C Auto-reject:	overage: 20 %]			
Modified Fragme Auto-reject:	Modified Fragments Requirement: Relaxed Auto-reject:				
IsoAsp Signature Auto-reject:	Ion Pair Required:				
0	OK	Can	cel	Apply	

• To automatically reject peptides that do not meet the specified criteria, select **Auto-reject**.

MS/MS Coverage tab:

- Min. Fragment Coverage: Enter a minimum peptide MS/MS coverage.
 - Peptides with MS/MS coverage below the threshold have a Q flag and the comment MS/MS coverage below XX% in the **Peptide Table** of *Review Results*.
- **Modified Fragments Requirement**: Select the level of MS/MS fragment ion evidence that is required to validate peptides with **Variable** modifications.
 - Strict: Requires evidence of MS/MS ions on both sides of a modified amino acid.
 - Variable modifications that do not meet this criterion have a **Q** flag and the comment Modified fragments (strict) not found in the **Peptide Table** of *Review Results*.
 - **Relaxed**: Requires evidence of MS/MS ions on <u>one side</u> of a modified amino acid.
 - Variable modifications that do not meet this criterion have a Q flag and the comment Modified fragments (relaxed) not found in the **Peptide Table** of *Review Results*.
 - **None**: Requires no evidence of MS/MS fragment ions on either side of a modified amino acid. Select this option to accept all modified peptides.

3	र्रे Fr	agment S	pectra Table X			
	Ŧ	$\overline{A} \in \widehat{A}$	👬 ¥ 🔲 ¥ 💼 ¥ 🍦 📲			
0		Group Id	Peptide	Score	Modifications	¹ Modified Fragments
		11755	TKPREEQYNSTYR	230.173	Man5 [N301]	Present (Strict)
	۲	14661	SLSLSPGK	95.374	Lys-loss [Protein C-term K]	Present (Strict)
		9693	EVQLVESGGGLVQPGR	95.60	l Glu->pyro-Glu [N-term E]	Present (Relaxed)
	٠	17365	EVQLVESGGGLVQPGR	129.687	' Glu->pyro-Glu [N-term E]	Present (Strict)
	۸	5970	TKPREEQYNSTYRVVSVLTVLHQDWLNGK	122.486	G2F [N301]	Present (Strict)

Modified peptides have either **Present** (Strict) or **Present (Relaxed)** in the **Modified Fragments** column in the **Fragment Spectra Table**.

Automatic Review: MS/MS Coverage



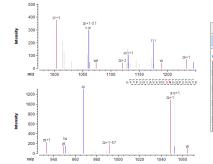


🐵 Automatic Revi	iew - Define Rules	- Settings		×
Isomer Det General	ection Modification RT Win	Crosslinks	D MS/MS Cov	isolav verage
Min. Fragment Co Auto-reject:	verage: 20 %]		
Modified Fragmen Auto-reject:	ts Requirement: [Relaxed		~
IsoAsp Signature	Ion Pair Required:			
Auto-reject:				
0 1	OK	Can	cel	Apply

 To automatically reject peptides that do not meet the specified criteria, select Auto-reject.

MS/MS Coverage tab:

- IsoAsp Signature Ion Pair Required: Select this option if peptides that contain Asp→IsoAsp or Deamidated (IsoAsp) modifications must have at least one of the signature fragment ion pairs (c and c+57, or, z and z-57) identified.
 - Signature ion pairs include all relevant c or z class ions, for example, z+1, z+1-57, z+1, z-57.
 - Peptides that do not meet this criterion have a Q flag and the comment IsoAsp signature ion pair not found in the Peptide Table of *Review Results*.
 - Relevant peptides have either **Present** or **Absent** in the **IsoAsp Signature Ion Pair** column in the **Fragment Spectra Table**.



LSCAASGFTFDDYAMHWV

C	₹ Fr	agment S	pectra Table X			
	т	Ţ €	🛄 ¥ 🛄 ¥ 🛄 ¥ 💼			
٥	۲	Group Id	Peptide	Score	Modifications	IsoAsp Signature Ion Pa
	٠	10285	VVSVLTVLHQDWLNGK	251.20	9 Deamidated (IsoAsp) [N319]	Present
	۰	11176	LSCAASGFTFDDYAMHWVR	143.01	4 Asp->IsoAsp [D31] Carbamidomethyl [C22 M34]	Present
	۰	11385	GFYPSDIAVEWESNGQPENNYK	374.12	5 Deamidated (IsoAsp) [N388]	Present
	٠	11901	GLEWVSAITWNSGHIDYADSVEGRFTISR	268.85	8 Asp->IsoAsp [D59]	Present
	٠	11964	LSCAASGFTFDDYAMHWVR = AEDTAVYYCAK	245.87	3 Asp->IsoAsp [D31] Carbamidomethyl [M34]; ;	Present
	۸	1044	TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK=EYKCK	126.99	3 Asp->IsoAsp [D269]; ;	Absent
	٠	1044	TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK=EYKCK	212.15	7 Asp->IsoAsp [D269]; ;	Absent
	٠	1044	TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK=EYKCK	94.26	0 Asp->IsoAsp [D269]; ;	Absent
		2037	SRWQQGNVFSCSVMHEALHNHYTQK	81.98	3 Carbamidomethyl [C429] Deamidated (IsoAsp) [N425]	Absent

Automatic Review: Isomer Detection



Automatic Review - Define Ru	es
------------------------------	----

Automatic	Review - Define Rules -	Settings	×
General Isome	Modification RT Wind r Detection	ows MS Crosslinks	/MS Coverage Display
Required Am	ino Acids:		
Ignore Modif	ied Peptides:		
0	ОК	Cancel	Apply

Isomer Detection tab:

- Select this option to add an I (Potential <u>I</u>somer) flag to peptides that have identical annotations but different Group IDs. This indicates the presence of potential isomers for manual review.
 - Required Amino Acids: Enter the single letter code for amino acids that must be in a peptide for it to be identified as an isomer candidate.
 - Ignore Modified Peptides: Select this option to ignore isomer candidates that contain modifications.

Automatic Review: Crosslinks



Automatic Review - Define Rules	ŝ
---------------------------------	---

Automatic Revi	ew - Define Rules - Settings	×
General N Isomer Dete	Modification RT Windows MS/MS Coverage action Crosslinks Display	
Flag Scramble	ed Crosslink Bonds	
Expected Bonds:	Disulfide[HC:22, HC:96] ^ Disulfide[HC:148, HC:204] Disulfide[HC:224, LC:214] Disulfide[HC:230, HC:230] Disulfide[HC:233, HC:233] Disulfide[HC:265, HC:325] v	
0	OK Cancel Apply	

Crosslinks tab:

- Select Flag Scrambled Crosslink Bonds to add a ≠ flag and the comment Contains unexpected crosslink bonds to the Peptide Table in *Review Results*.
 - Expected Bonds: Use the correct syntax for the known disulfide bonds.
 Enter the type of crosslink, and then the protein and amino acid position for each bond.
 - For example: Disulfide[HC:22, HC:96] or Trisulfide[HC:371, LC:194]
 - The protein names must be identical to those in the **Sequence** tab.
 - Different crosslinks cannot contain the same location.

Note: In Biologics Explorer Software 5.0, peptide mapping results of crosslinked peptides with **Modification RT Windows** flags should be manually assessed. **Auto-Reject** should not be selected.

Review Results: Configure Settings





🐵 Review Resul	ts - Settings		×
Peptid	e Chromatograms Modifications	Report Crosslinks	Display Conjugates
Sequence(s):	From Data		\sim
0	ОК	Cancel	Apply

Sequence tab:

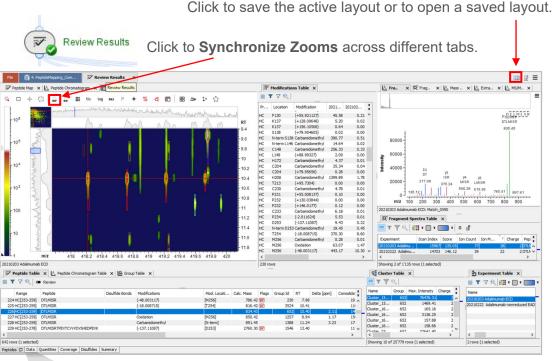
- Sequence(s):
 - To use the sequence information specified in the previous *Peptide Mapping* activity nodes, select **From Data**.

🐵 Review Results - Setti	ngs		×
	natograms Iodifications	Report Crosslinks	Display Conjugates
Fixed: Carbamidomethy	I (C)		+
0	OK	Cancel	Apply

Modifications tab:

• Select the **Fixed** modifications specified in the previous *Peptide Mapping* activity nodes.

Review Results: Create Custom Layouts



des Data Click to undock the **Data** tab window.

- Each pane can be undocked and then docked at a new location.
- The location where the pane will be docked is highlighted by a blue box.
- To move useful tables and visualizers to the **Peptides** tab:
 - 1. To undock the **Data** tab, click the 🗖 icon.
 - 2. To undock any pane from the **Data** tab window, drag it to a new location on the **Peptides** tab.
- Favorite layouts can be saved and opened with the **Layout** icon.

Note: For more information, refer to the document: Biologics Explorer Software Quick Guide.

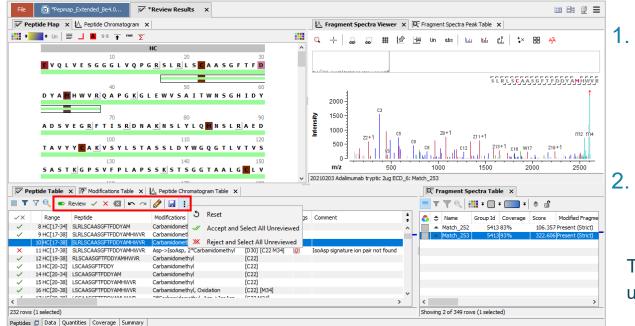


Review Results: Review Peptide Mapping Results





Open the *Review Results* activity node to review the combined results of the preceding *Peptide Mapping* activity nodes.



- . Activate the **Review** mode, and then **Accept** or **Reject** annotations.
 - To add a comment, either type in the applicable row in the Comment column, or use the *icon* to add the same comment to multiple rows.
- To apply the changes, click the **Save** icon, and then select **Save and Reload**.

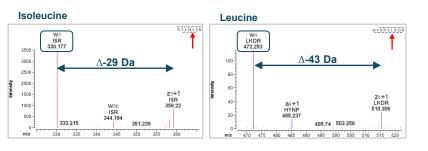
The activity node then automatically updates the peptide **Quantities** table.

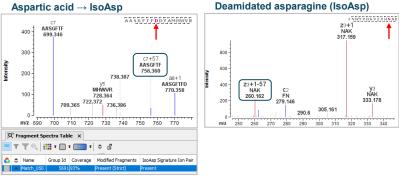
Review Results: Isomer Differentiation





 MS/MS analysis with EAD creates diagnostic internal fragment ions that help to identify different isomeric amino acid residues.





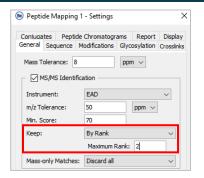
- Leucine (Leu) or isoleucine (IIe):
 - lons are annotated as: w_n or w_{nb}.
 - Leucine: w_n ion at a 43 Da mass shift from the corresponding z (or z+1) ion.
 - Isoleucine: w_n ion at a 29 Da mass shift from the corresponding z (or z+1) ion.
 - Isoaspartic acid (IsoAsp):
 - lons are annotated as: c_n +57 or z_m -57 (or z_m +1-57).
 - c_n +57 or z_m -57 ions in the MS/MS spectra identify isoaspartic acid.
 - Aspartic acid does not have these diagnostic internal fragment ions because the peptide backbone does not have a methylene group.

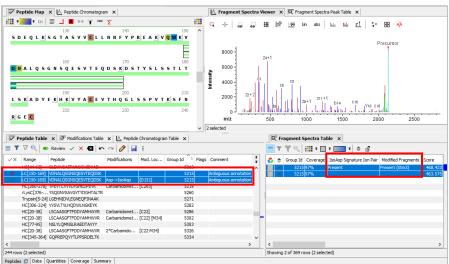
Review Results: Isomer Differentiation





- To see alternative identifications for the same peptide, use Keep: By Rank and Maximum Rank: 2 in the settings for Peptide Mapping.
- For data review in the **Peptide Table** in *Review Results*:
 - 1. Click the **Group Id** column header to order entries numerically. Ambiguous annotations have the same **Group Id**.
 - Select **Detect Ambiguous Annotations** in *Automatic Review*, to add information to the **Comment** column.
 - 2. Use the diagnostic fragment ions in the MS/MS spectra to validate identifications.
 - To add information about diagnostic fragment ions to the Comment column in the Peptide Table, select IsoAsp Signature Ion Pair Required in Automatic Review.
 - 3. Select Accept or Reject for each result as required.
 - 4. To apply the changes, click the **Save** icon, and then select **Save and Reload**.



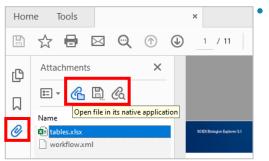


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Export and Report Results



	Export PDF Report (20	Save Resu	lts Sr	iapshot
6	Export to SCIEX O	S			
	👤 File System				
	3 💿 🖻 🏂	🏂 Upload	Files Downloa	ad Files	
	🛅 Home (Personal)/PepMa	p_Extended			
	Name			Size	
	Reports			_	
	SCIEX_OS				



There are three types of activity nodes to report or export results at the end of a workflow:

- Save Results Snapshot: Reviewed results are saved as sbf files that can be opened in the Pepmap_ReviewSnapshots workflow.
- **Export PDF Report**: A summary of results is saved as a PDF.
- **Export to SCIEX OS**: Results are saved as txt files that can be used for applications that use the SCIEX OS software.
- Select or add the folders where the results will be stored.
- The exported PDF Report includes:
 - A PDF document.
 - An embedded Excel file.
 - An embedded workflow file (xml) that includes all of the settings.
 - To open the xml file, drag the saved PDF Report into the workflow home page in the Biologics Explorer software.

Note: For more information, refer to the document: Biologics Explorer Software Quick Guide.

Export Intermediate Results for Further Analysis



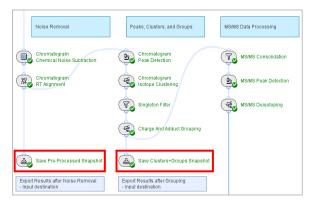
- The Save Snapshot activity nodes save intermediate results at different stages of a workflow.
 - An individual sbf file is saved for each sample processed in the workflow.
 - The sbf file contains the properties of the processed data that are required to continue analysis from that point in the workflow.
- To use a *Save Snapshot* activity node to save intermediate results:
 - Deactivate the **Block** icon.
 - Select or add the folders where the sbf files will be stored.



Save Data Preparation Snapshot



Use Intermediate Results for Further Analysis



🐵 Load Ra	aw Data - Setti	ngs X	
General A	dvanced Disp	lay	
Name:	Peptide I	lapping	
Format:	Snapsho	t (*.sbf) ~	
Files/Fold	202102	03 Adalimumab trybtic 2ug ECD_4.sbf 03 Adalimumab trybtic 2ug ECD_5.sbf 33 Adalimumab trybtic 2ug ECD_6.sbf	£
	3 items	 ♣ Name ▲ 20210203 Adalimumab tryptic 2ug ECD 4 	Source Type
		20210203 Adaimumab tryptic 20g ECD_4 20210203 Adaimumab tryptic 2ug ECD_5 20210203 Adaimumab tryptic 2ug ECD_6	SCIEX WIFTWO SCIEX WiffTwo SCIEX WiffTwo

- To continue data analysis with saved Snapshots, such as those from in the *Data Preparation [Container]*:
 - 1. Open a suitable Peptide Mapping workflow.
 - 2. Select the sbf files to import into *Load Raw Data*.
 - 3. Change the Format to Snapshot (*.sbf) or Auto Detect.
 - 4. Activate the **Bypass** icon on the activity nodes in the workflow that are before the Snapshots were saved.
 - For example: To load sbf files from *Save Clusters+Groups Snapshot*, activate the **Bypass** icon for all activity nodes between *Load Raw Data* and *MS/MS Consolidation*.

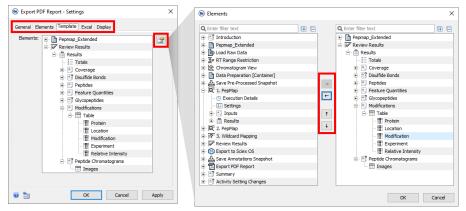


Export PDF Report



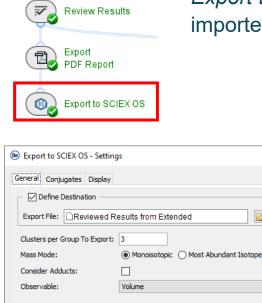


- The output of *Export PDF Report* includes:
 - A PDF document.
 - An optional Excel file.
 - An embedded workflow file (xml) that includes all of the settings.
 - To open the xml file, drag the saved PDF Report into the workflow home page in the Biologics Explorer software.
 - Note: For more information, refer to the document: Biologics Explorer Software Quick Guide.
- **General** tab: Enter the name and saved location of the exported report.
- **Template** tab: Use the **Edit Selection** icon to select the **Elements** to be included in the report.
 - Select only columns of interest in reported tables. The layout of the tables is controlled by the number of columns.
- Excel tab: Use the Edit Selection icon to select the Tables to be included in the report.
 - All columns in a selected table are reported.



Export to SCIEX OS





OK

Cancel

Export to SCIEX OS creates a txt file of peptide mapping data that can be imported into the SCIEX OS software for further data processing.

> General tab:

 \times

2

 \sim

Apply

- Enter the name and saved location of the exported report.
- Select the requirements of the export, for example, the number of clusters for each group, and if adducts are to be included.
- Use **Observable: Volume** for data acquired using a QTOF mass spectrometer.

Note: The modification position exported with the *Export to SCIEX* OS activity node is relative to the peptide, not the protein. For example, DTL[M]ISR would be M4, not M255.

- The *Export to SCIEX OS* activity node should not be used in combination with the *Wildcard Mapping* activity node.
 - Activate the **Bypass** icon on the *Wildcard Mapping* activity node when required.

🕜 🛅

Part B Guidelines for Specific Peptide Mapping Workflows



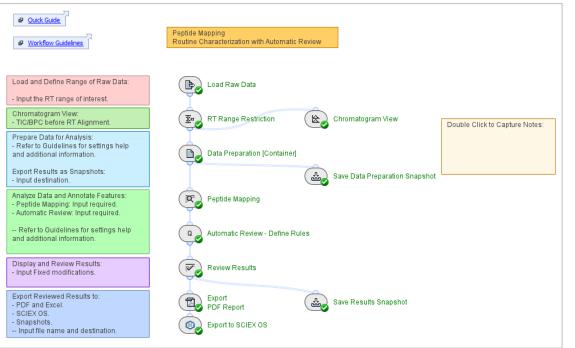
Comments of

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1. Simple Peptide Mapping

WORKFLOW SPECIFIC INFORMATION AND GUIDELINES

Simple Peptide Mapping Workflow



- Use this workflow for routine analysis of non-complex biotherapeutic molecules.
- Edit the combination of search parameters in the *Peptide Mapping* activity node to identify peptides and modifications, including glycosylation.

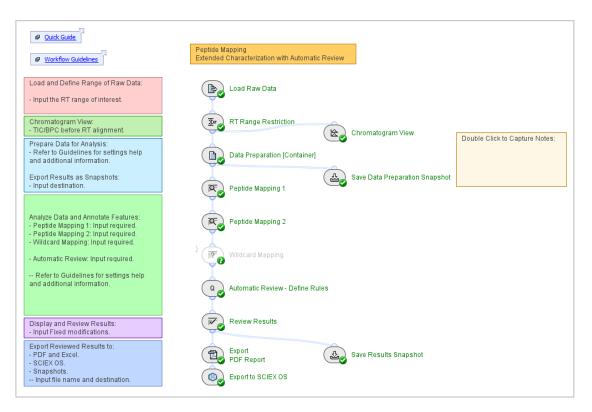
Pepmap_Simple_Be5.0



2. Extended Characterization

WORKFLOW SPECIFIC INFORMATION AND GUIDELINES

Extended Peptide Mapping Workflow



• Use this workflow for a more comprehensive Peptide Mapping analysis of biotherapeutic molecules.

The Power of Precision

• Combine results from up to three consecutive *Mapping* activity nodes to extend the search space and increase identifications, but keep false positives to a minimum.



Extended Characterization





Peptide Mapping 2	- Settings	×		
Coniugates I General Sequence		Report Display lation Crosslinks		
Mass Tolerance: 8	ppm ~			
Instrument:	EAD ~			
m/z Tolerance: Min. Score:	50 ppm ~			
Keep:	Top Ranked	~		
Mass-only Matches:	Discard all	~		
☐ Ignore Annotated Features Export Coverage Data (deprecated)				
0 🛅	OK Cancel	Apply		

This workflow uses up to three consecutive search nodes to extend the search space but minimize false positives:

Peptide Mapping 1

- To identify the most likely peptides and modifications.

Peptide Mapping 2

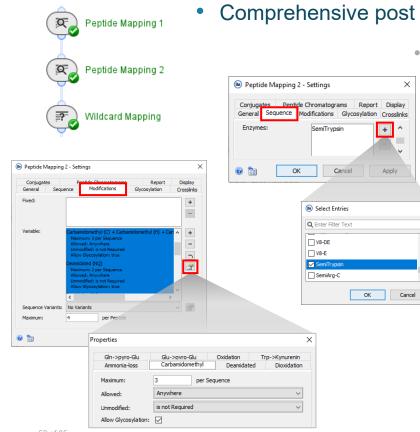
- To search for less common modifications.
- Ignore Annotated Features: Makes sure that only unannotated features from the previous search are considered.
 - **Example Use Case**: For biotherapeutics with *N* and *O*-glycosylation, false positives are decreased when *Peptide Mapping 1* is used to identify *N*-glycans, and *Peptide Mapping 2* is used to identify *O*-glycans.

Wildcard Mapping

- To search for unexpected modifications.
 - Add identified modifications to a *Peptide Mapping* activity node.
 - Activate the **Bypass** icon when *Wildcard Mapping* is not required.

Step-Wise Peptide Mapping: Application Example





- Comprehensive post translational modification (PTM) analysis.
 - Suggested settings for this type of analysis:

Peptide Mapping 1

- Sequence tab. Enzymes: Fully specific (Trypsin).
- Modifications tab: Abundant and most likely modifications.

Peptide Mapping 2

- Sequence tab. Enzymes: Semi-specific (SemiTrypsin).
- **Modifications** tab: Shorter list of most likely modifications. Or:
- Sequence tab: Enzymes Fully specific (Trypsin).
- **Modifications** tab. Alternative set of less common modifications that might be present at low abundance.

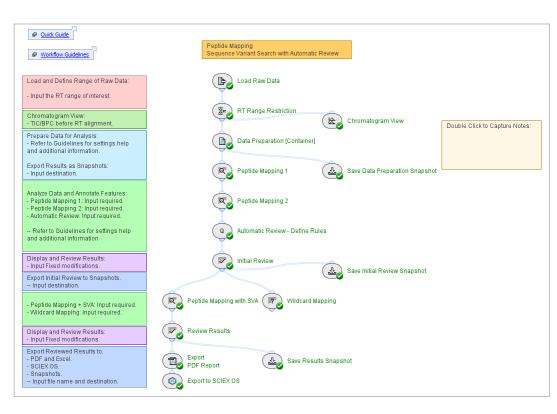
Wildcard Mapping

• Select **All Peptide Candidates** for annotations related to unknown modifications.

3. Sequence Variant Search

WORKFLOW SPECIFIC INFORMATION AND GUIDELINES

SVA Peptide Mapping Workflow



• Use this workflow to detect potential sequence variants.

The Power of Precision

- Note: High quality MS and MS/MS data is required for confident identification of sequence variants. Instrument acquisition should be optimized for SVA.
- Two consecutive *Peptide Mapping* activity nodes identify the non-variant peptides and remove them from the search space.
 - Note: For more information about step-wise Peptide Mapping, refer to the section: B: 2.Guidelines for Extended Peptide Mapping Workflows.
- A further *Peptide Mapping with SVA* activity node searches for sequence variants.
- Possible SVA identifications can be compared with the results from the *Wildcard Mapping* activity node for verification.

SVA Peptide Mapping Workflow

SCIEX The Power of Precision

To detect potential sequence variants:

- 1. Use *Peptide Mapping 1* and *Peptide Mapping 2* to complete a typical analysis for non-variant peptides.
 - Refine the *Peptide Mapping* settings for the molecule under investigation.

Note: For more information about step-wise Peptide Mapping, refer to the section: **B**: 2.Guidelines for Extended Peptide Mapping Workflows.

- \rightarrow Identified peptides are removed from the search space, which decreases false positives in the next stage.
- 2. Complete an initial review of the data.
 - Click the Save icon, and then click Save and Reload.
 - \rightarrow Features from the rejected annotations are considered again in the *Peptide Mapping with SVA* search.



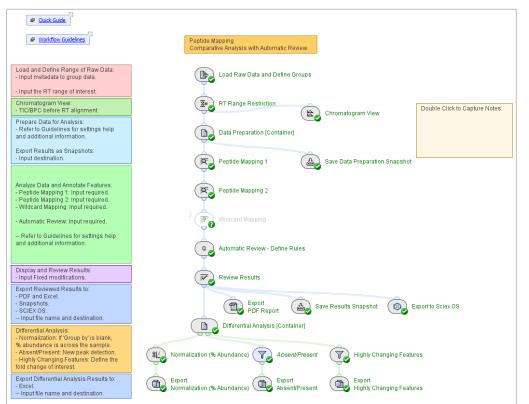
- 3. Deactivate the **Pause** icons on the *Peptide Mapping with SVA* and *Wildcard Mapping* activity nodes, and then click the **Play** icon to run them.
 - To increase the number of possible identifications, decrease the **Min. Score** in *Peptide Mapping with SVA* and *Wildcard Mapping*. A lower **Min. Score** increases the false positives for review. Manually review identifications that are close to the **Min. Score** threshold to reject incorrect annotations
- 4. Compare identifications in *Peptide Mapping with SVA* and *Wildcard Mapping*.
- 5. Review, and then Accept and Reject entries in the Peptide Table as required.

Note: For settings information for identification of low abundance peptides, refer to the pages: *MS/MS Peak Detection* and *MS/MS Deisotoping* in **A**: 2.General Guidelines for Peptide Mapping Workflows.

4. Comparative Analysis

WORKFLOW SPECIFIC INFORMATION AND GUIDELINES

Comparative Peptide Mapping Workflow



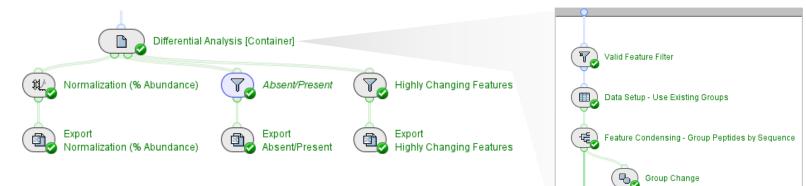
Pepmap Comparative Be5.0

- Use this workflow to compare two sets of samples, for example:
 - Reduced and non-reduced samples.
 - Stressed and unstressed samples.
 - A reference sample with samples from a new batch.
- Combine results from up to three consecutive *Mapping* activity nodes.
 - Note: For more information about step-wise Peptide Mapping, refer to the section: B: 2.Guidelines for Extended Peptide Mapping Workflows.
- The *Differential Analysis [Container]* prepares data for comparisons to be made.



Comparative Peptide Mapping

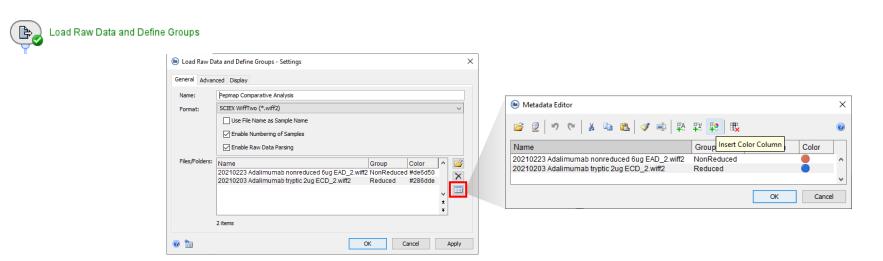




The statistical activity nodes identify features that differ significantly between the two sample groups being compared in the workflow.

- The activity nodes connected with green lines contain statistical tools that can be used to compare two datasets.
- The workflow reports:
 - The relative (%) abundance of peptides in each dataset.
 - The peptides that are absent in one sample set, but present in the other.
 - The peptides that have a specified fold-change difference between sample sets.

Load Raw Data and Define Groups



- Click the table icon to open the **Metadata Editor**.
 - Enter the **Group** names for the files to be compared.
 - Optionally, add a **Color** column and enter a color for each group.

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Application Example 1: Disulfide Bond Analysis



Peptide Mapping 1	 Comparative analysis 	s of reduced and non-reduced samples.
Peptide Mapping 2	0	Recommended settings for this type of a
Wildcard Mapping 2	Peptide Mapping 1 - Settings Conjugates Peptide Chromatograms Report Disulay Connectivity De Novo Max. Peptides: 2 Crosslinker: Disulfide [Cys-Cys Loss: Ha] Thioether [Cys-Cys Loss: Ha] Trisulfide [Cys-Cys Loss: Ha] Trisulfide [Cys-Cys Loss: Ha] Trisulfide [Cys-Cys Loss: Ha] Trisulfide [Cys-Cys Gain: S Loss: Ha] Trisulfide [Cys-Cys Gain: S Loss: Ha] Conjugates Peptide Mapping 2 - Settings X Conjugates Peptide Chromatograms Report Disolay Connectivity None	 Peptide Mapping 1 Sequence tab. Enzymes: Fully specific (Tr. Disulfide tab. Connectivity: De Novo Modifications tab. Variable alkylation of cy Peptide Mapping 2 Sequence tab. Enzymes: Semi specific (Set Disulfide tab. Connectivity: None. Wildcard Mapping Select All Peptide Candidates for more an unknown modifications.

- gs for this type of analysis:
 - mes: Fully specific (Trypsin).
 - ectivity: De Novo
 - /ariable alkylation of cysteine.
 - mes: Semi specific (SemiTrypsin).
 - ectivity: None.
 - andidates for more annotations related to ns.

Note: For more information about step-wise Peptide Mapping, refer to the section: B: 2. Guidelines for Extended Peptide Mapping Workflows.

Application Example 2: Stress Tests and Variability



Peptide Mapping 1	• Coi	mparativ	e analys
	Peptide Mapping 2	- Settings	×
Peptide Mapping 2	Conjugates P General Sequence	eptide Chromatograms Modifications Glyc	Report Display osylation Crosslinks
Wildcard Mapping	Fixed:		+
Ŷ		Ammonia-loss (N) Maximum: 1 per Sequence Allowed: Anywhere Unmodified: is Required Max. Distance: 2.0 Minut Same Charge: true Allow Glycosylaton: true Carbamidomethyl (C) + Ca Maximum: 3 per Sequence Allowed: Anywhere	
	Sequence Variants:	No Variants	
	Maximum:	3 per Peptie	le
Properties		or ca	Apply
Gln->pyro-Glu		Dxidation Trp->Kynur	
Ammonia-loss	Carbamidomethyl	Deamidated Dioxid	ation
Maximum:	3 per Sequ	ence	
Allowed:	Anywhere		\sim
Unmodified:	is not Required		~
Allow Glycosylation	is not Required is Required		
	must be Absent		

is for stress testing and lot-to-lot variability.

Recommended settings for this type of analysis: Peptide Mapping 1

- Sequence tab. Enzymes: Fully specific (Trypsin). ٠
- **Modifications** tab. Abundant and most likely modifications. •

Peptide Mapping 2

- Sequence tab. Enzymes: Semi specific (SemiTrypsin). ٠
- **Modifications** tab. Shorter list of most likely modifications. •

Or:

- Sequence tab. Enzymes: Fully specific (Trypsin). ٠
- Modifications tab. Alternative set of less common modifications . that might be present at low abundance.

Wildcard Mapping

Select **All Peptide Candidates** for annotations related to • unknown modifications.

Note: For more information about step-wise Peptide Mapping, refer to the section: **B**: 2.Guidelines for Extended Peptide Mapping Workflows. © 2024 DH Tech, Dev. Pte. Ltd.

Differential Analysis [Container]

Differential Analysis [Container]

Feature Condensing - Group Peptides by Sequence

Valid Feature Filter

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Data Setup - Use Existing Groups

Group Change

1

-E)



- Valid Feature Filter
 - Removes any features below a set threshold, and any found in less than a set % or number of experiments.
 - Removes insignificant differences or signal caused by noise or artifacts. If predicted peptides are not found, then optimize this setting.
 - Data Setup
 - Prepares data in a matrix form for the next activity nodes.
 - If groups were not defined in *Load Raw Data*, use Group: Manually and assign each sample to a group.
 - Feature Condensing
 - Uses annotations to group features.
 - Calculates a single intensity value for each of the created groups.
 - Group Change
 - Calculates relative and fold-change differences between experiments.
 - If there are multiple experiments, then the reported change is the maximum difference between any two experiments.

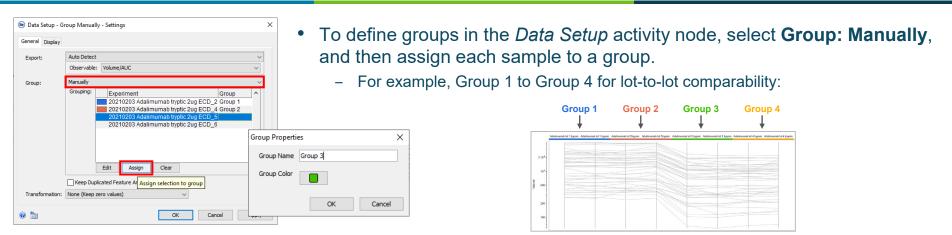
Note: Available settings are related to the previous activity nodes. To see the list of possible **Group by** options, run *Data Setup*, and then edit the *Feature Condensing* settings.

Differential Analysis [Container]: Data Preparation

ame [1]

ande

eptide odifications



Beature Condensir	•					
General Display						
Group by:	Protein Peptide Modifications					
Condensing Method:	nod: Sum 🗸					
Group Ordering:	Existing Order Alphabetical					
	Keep Ungrouped					
	Keep Duplicated Feature Annotations					
0	OK Cancel	Group by:				
			✓ P			

- To use annotations to combine the features in each group, and calculate a single intensity value for the combined feature, select the types of annotations to **Group by** in *Feature Condensing*.
 - For example, to combine peptides with the same sequence and modifications, from the same protein, select: **Protein**, **Peptide**, and **Modification**.

Note: To see the list of possible **Group by** options, run *Data Setup*, and then edit the *Feature Condensing* settings.

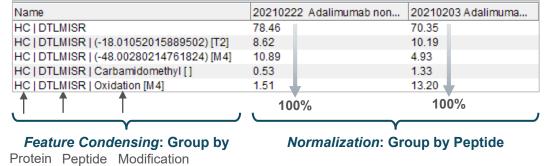
The Power of Precisio

Normalization (% Abundance)



Normalization (% Abundance) - Settings						
General Display						
Normalization:	Percent Abu	undance	~			
	Group by:	\sim				
0 1	ОК	Cancel	Apply			

- Normalization (% Abundance)
- Percent Abundance: For each experiment, the values of all members of each group are added. Then, the value of each member of a group is divided by the corresponding total and multiplied by 100.
 - If Group by is set to Peptide, then the Percent Abundance is calculated for each peptide.
 - If Group by is blank or set to Undefined, then the Percent Abundance is calculated for the whole sample.

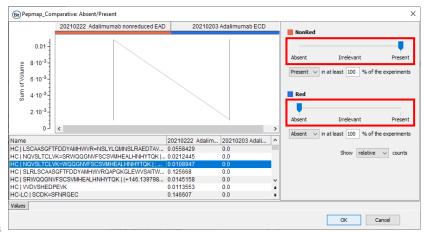




Absent/Present and Highly Changing Features



- Absent/Present
 - Compare features between two sample groups.
 - To filter the results, move the sliders in the input window _ that opens when the activity node is run.
 - For example, for disulfide bond analysis, the features of interest are likely to be Absent in the reduced sample and Present in the nonreduced sample.



Highly Changing Features

Set the required minimum fold change.



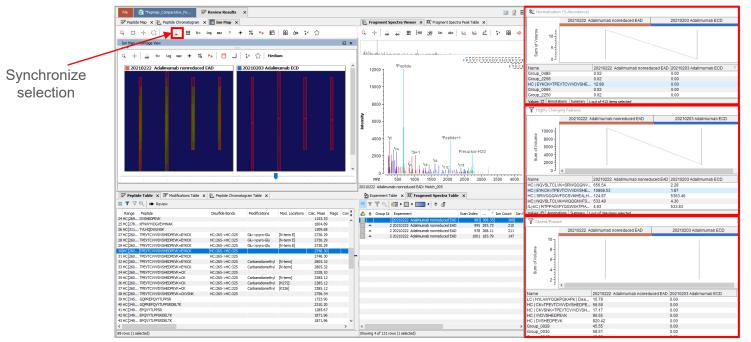
File 🗐 '	4. PeptideMappir	ng_Com 🛛 🍸 Fold chan	ge >10 🗙 📰 📑	: 2 ≡	File	*4. PeptideMapping_C	η	Fold change >1	0 × 🔳 📑	¥ =
	0222 Adalim	numab nonreduced EAD	20210203 Adalim	umab ECD	Name		Color Gr	oup Method N	ame	12
40000 - 20000 -						: Adalimumab nonreduc Adalimumab ECD	No		IDA most intense 90r IDA most intense 90r	
0 -	<			>		None selected-	/			
Name	20210222	Adalimumab nonreduced	EAD 20210203 Adalimu	mab 🔺	Name	Disulfide Bond	ls v	Group Change	Group Change [Abso	olute]
Group_02955	109.43		0.00		Group_02	955 LC:88->LC:23		0.00	00	
Group_01847	25.79		0.00		Group_01	847 LC:23->LC:88		0.00	00	
Group_02240	7.18		0.00		Group_02	240 LC:23->LC:88		0.00	00	
Group_00020	1246.17		0.22		Group_00	020 LC:23->LC:88		0.00	5633.46	
Group_00292	157.25		0.03		Group_00	292 LC:23->LC:88		0.00	5141.97	
Group_00198	2871.66		1.82		Group_00	198 LC:23->LC:88		0.00	1578.17	
Group_00006	1616.91		1.43		Group_00	006 LC:23->LC:88		0.00	1131.01	
Group_00120	397.96		0.43		Group_00	120 LC:23->LC:88		0.00	925.83	
Group_00049	1267.23		2.88		Group_00	049 LC:23->LC:88		0.00	439.58	
Group_00045	13010.14		31.26		Group_00	045 LC:23->LC:88		0.00	416.17	
Group_00022	337.01		0.85		Group_00	022 LC:23->LC:88		0.00	397.29	
Group_02945	14.23		0.05		Group_02	945 LC:23->LC:88		0.00	289.84	
Group_01912	24.60		0.16		Group_01	912 LC:23->LC:88		0.01	158.67	
Group_00804	172.31		1.40		Group_00	804 LC:23->LC:88		0.01	122.77	
Group_00046	1089.63		9.21		Group_00	046 LC:23->LC:88		0.01	118.33	
Group_00116	69.05		1.52		Group_00	116 LC:23->LC:88		0.02	45.28	
Group_00160	30.89		0.87		Group_00	160 LC:23->LC:88		0.03	35.37	



Synchronize Selections for Simplified Data Review



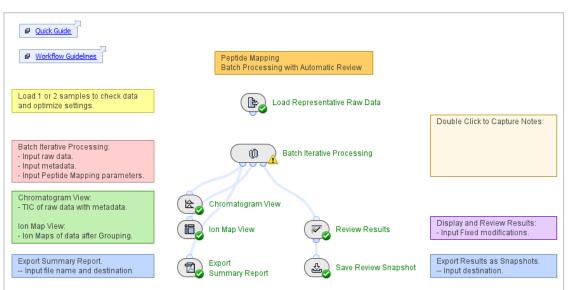
- To automatically update (dynamically link) the selection in all results windows:
 - 1. Open the results windows for *Absent/Present*, *Highly Changing Features* and *Review Results*.
 - 2. Click the Synchronize selection icon in Ion Map tab of Review Results.



5. Peptide Mapping Batch Processing

WORKFLOW SPECIFIC INFORMATION AND GUIDELINES

Peptide Mapping Batch Processing Workflow



Pepmap_BatchProcessing_Be5.0

- Use this workflow to analyze multiple samples independently, in an iterative manner.
- Use the Pepmap_Extended workflow to optimize the workflow settings for any new samples before running a Batch Processing workflow.
 - Note: For more information about step-wise Peptide Mapping, refer to the section: B:
 2.Guidelines for Extended Peptide Mapping Workflows.
- Use the Pepmap_ReviewSnapshots workflow to review data and create reports for samples analyzed with the Batch Processing workflow.



Recommendations for the Batch Processing Workflow



Load Representative Raw Data

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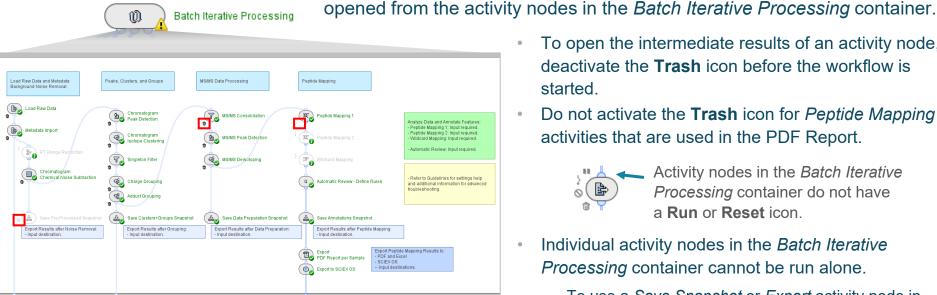


Export Results after Peptide Mapping: - Input destination.

- To use the Batch Processing workflow:
 - I. Use the *Load Representative Raw Data* activity node to first review a small number of representative samples.
 - Examine the RT range and noise level.
 - Use the Pepmap_Extended workflow to optimize the workflow settings for any new samples.
 - 2. Use a small *RT Range Restriction*, for example 10 min to 15 min, and a smaller sample set, so that the Batch Processing workflow contains all applicable metadata and completes successfully.
 - To monitor progress, double-click the icon to open the Log Messages dialog.
 - 3. Use the Save Annotations Snapshot or Save Review Snapshot activity node to save Peptide Mapping results. Load each saved sbf file separately in the Pepmap_Review Snapshots workflow to review the results and create individual PDF reports.

Batch Iterative Processing Container

 The Batch Iterative Processing container is not the same as other Biologics Explorer software containers. Only intermediate results from the last sample to be processed can be



Note: If activity nodes in the container have the **Bypass** icon activated, then the container shows a yellow warning symbol.

- To open the intermediate results of an activity node, deactivate the Trash icon before the workflow is started.
 - Do not activate the **Trash** icon for *Peptide Mapping* activities that are used in the PDF Report.
 - 0
- Activity nodes in the *Batch Iterative Processing* container do not have a Run or Reset icon.

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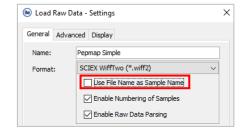
- Individual activity nodes in the Batch Iterative *Processing* container cannot be run alone.
 - To use a Save Snapshot or Export activity node in _ the Batch Iterative Processing container, deactivate the **Block** icon before the workflow is started.

Load Raw Data: Experiment Names and Metadata



- To analyze replicate samples from different acquisition files:
 - . Select Use File Name as Sample Name in Load Raw Data.
 - 2. Use the **File Name** (name of the wiff or wiff2 container file) in the **Experiment** column of the txt file for *Metadata Import*.
- To analyze multiple samples from a single acquisition file:
 - 1. Do not select Use File Name as Sample Name in Load Raw Data.
 - 2. Use the **Sample Name** in the **Experiment** column of the txt file for *Metadata Import*.

	Chared/Raw_Data/Pepmap_	raw/CID	
	Name	Eile Eile	
	🖻 NIST mAb digest IDA EB	03 100ng on column R01.wiff2	ename
8	Shared/Paw Data/Penman raw/CID	NIST mAb digest IDA EB03 100ng on column R01.wiff2	
Na		NIST MAD digest IDA ED05 100hg on column K01.Win2	
D	Peptide mapping CE_4 R01 [1]		For more
	Peptide mapping CE_6 R01 [2] Peptide mapping CE_5 R01 [3]	Sample names	Name as
	Peptide mapping CE_7 R01 [4] Peptide mapping CE_8 R01 [5]	•	Mapping



For more information, refer to the page: *Load Raw Data*: Use File Name as Sample Name in **A:** 2.General Guidelines for Peptide Mapping Workflows.

Load Raw Data

Metadata Import

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Metadata Import



B	Load Raw Data
	Metadata Import

- To analyze multiple samples with the <u>same sequence</u>:
 - Deactivate the **Trash** icon, and then activate the **Bypass** icon for *Metadata Import*.
 - On the **Sequences** tab in *Peptide Mapping*, select **From Text** or **From Fasta File**.
- To analyze multiple samples with different sequences:
 - Use *Metadata Import* to select the FASTA file (protein sequence) that will be used for identification in the *Peptide Mapping* activity nodes.
- Upload a txt file with *Metadata Import* that links each sample to the correct FASTA file.
 - The name in the **Experiment** column must be the same as in the **Experiment** table in *Load Raw Data*.
 - The name in the Fasta File column must be the same as the name of the FASTA file that is in the specified Fasta File Directory, including the file extension (fasta or txt).

	А	В			1	
1	Experiment	Fasta File	Sequence(s):	From Metadata: Fasta File, Sequence IDs (optional) \sim		Name
2	20210203 Adalimumab tryptic 2ug ECD_1	Adalimumab.fasta		Define Fasta File Directory		🕅 Adalimumab.fasta
3	20210130 Herceptin IDA ECD Most intense _1	Herceptin.fasta		Directory: Cafasta		🕅 Herceptin.fasta
4	20210130 Rituximab IDA ECD Most intense _1	Rituximab.fasta				🕅 NIST.fasta
5	20210901_NISTmAb_TimeCourse_Control_4ul_EAD_1	NIST.fasta				🕅 Rituximab.fasta

Note: For more information, refer to the page: *Review Results*: Protein Name in FASTA Files in **B**: 5.Guidelines for Peptide Mapping Batch Processing Workflows.

Metadata Import: How to Create the Metadata File



	Metadata	Import					
	Files/Folders:	20210130 R	dalimumab ti lerceptin IDA ituximab IDA VISTmAb_Tir	ECD Most ECD Mos	t intense _* t intense _	1.wi 1.w 🗙	(2)
Metadat	a Editor						×
€ 2	<i>v c</i> ⊁	B B	🗸 🗉 🏗	F # F ®	II,		۲
lame					Group	Description	
0210130	Adalimumab try Herceptin IDA E Rituximab IDA F	CD Most inten	ise_1.wiff2		(3)		^

Note: Any metadata added in the *Load Raw Data* **Metadata Editor** table must be completed for all rows (all samples).

130 Rituximab IDA ECD Most intense _1.wiff2 201 NISTmAb TimeCourse Control 4ul FAD 1 w

Experiment	(4)	Fasta File
20210203 Adalimumab tryptic 2ug ECD_1	(-)	Adalimumab.fasta
20210130 Herceptin IDA ECD Most intense _1		Herceptin.fasta
20210130 Rituximab IDA ECD Most intense _1		Rituximab.fasta
20210901 NISTmAb TimeCourse Control 4ul	EAD 1	NIST.fasta

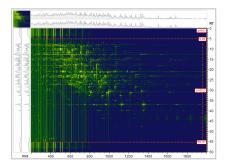
- To create the metadata file in Excel or Notepad:
 - 1. Select the samples for batch processing in *Load Raw Data*.
 - 2. Open the **Metadata Editor** table.
 - 3. Select all of the entries in the **Metadata Editor** table, and then select copy.
 - 4. Paste the entries into the **Experiment** column of the metadata txt file.
 - Delete ".wiff" or ".wiff2" from the end of each name. (Tip: Use the Replace command in Excel or Notepad.)
 - 5. Type the applicable FASTA file name in each row in the **Fasta File** column.
 - 6. Save the file in txt format, and then upload the file in the *Metadata Import* activity node.

Restrict the RT Range



🕞 🐊 Load Representative Raw Data 🛛

- Use the Load Representative Raw Data activity node to review a small number of representative samples outside of the Batch Iterative Processing container.
- To identify the RT ranges where there is meaningful data, open (double-click) *Load Representative Raw Data* after the data is loaded.
- If the RT ranges are consistent across all samples, then deactivate the Bypass icon and enter RT Minimum and RT Maximum values in the RT Range Restriction activity node in the Batch Iterative Processing container.







RT Range Restriction - Settings							
General Displa	1						
RT Minimum:	5	Minutes					
RT Maximum:	55	Minutes					
0 🖿 📃	ОК	Cancel	Apply				

Note: If the fields are blank, or if *RT Range Restriction* has the **Bypass** icon activated, then the full RT range is used.

Peptide Mapping: Sequence tab



Peptide Mapping 1 - S	Settings		>
Conjugates P	eptide Chromatograms	Report	Display
General Sequence	Modifications	Glycosylation	Crosslinks
Sequence(s):	From Text		~
	From Text From Fasta File From Protein Configura	ation File	
	From Global File From Metadata: Fasta	File Coguence I	De (ention
	From Metadata: Pasta		DS (Option)
Enzymes:	Trypsin		+
Max. Missed Cleavages:	4		
Min. Peptide Length:	5		
) 🎦	OK	Cancel	Apply

Sequence tab:

- Sequence(s):
 - If all samples have the <u>same sequence</u>, then select **From Text** and type the sequence, or **From Fasta File** and select the applicable file.
 - If different samples require <u>different sequences</u>, then select From
 Metadata: Fasta File, Sequence IDs (optional), and then browse to the location of the folder that contains all of the applicable FASTA files.

Sequence(s):	From Metadata: Fasta File, Sequence IDs (optional) $$
	Define Fasta File Directory
	Directory: 🖻 fasta

Enzymes:

 Adjust enzyme specificity, maximum number of missed cleavages, and minimum peptide length as required.



Review Results: Protein Name in FASTA Files



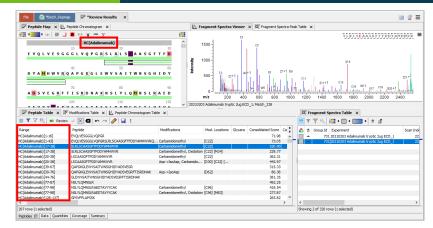
• If the protein sequence names <u>are unique</u> across the FASTA files used for identification:

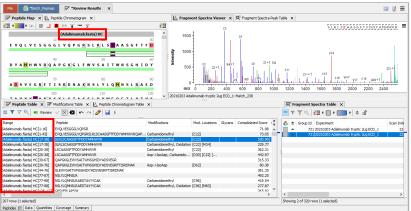
adalimumab.fasta - Notepad	
File Edit Format View Help	
>HC(Adalimumab)	
EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDY/	DSVEGRFTI
>LC(Adalimumab)	FEEEEEE
DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPS	IFSGSGSGTD
🛄 *Trastuzumab.fasta - Notepad	
File Edit Format View Help	
>HC(Trastuzumab)	
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYA	DSVKGRFTI
>LC(Trastuzumab)	

- The protein sequence name in *Review Results* is the same as the name in the FASTA file.
- If the protein sequence names <u>are not unique</u> across the FASTA files used for identification:

Adalimumab.fasta - Notepad
File Edit Format View Help
>HC EVQLVESGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEGRF >LC
DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSG
Trastuzumab.fasta - Notepad
File Edit Format View Help
>HC
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFT
>LC
DIOMTOSPSSLSASVGDRVTITCRASODVNTAVAWYOOKPGKAPKLLIYSASFLYSGVPSRFSGSRSG

 The protein sequence name in *Review Results* includes the FASTA file name.





Export PDF Report



• There are two locations to save a PDF Report in the Batch Processing workflow:



Export Results	s Summary Report.

-- Input destination.





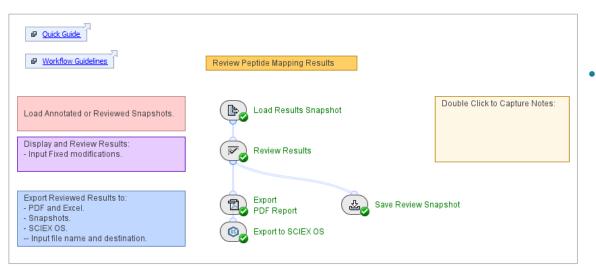
- 1. Export PDF Report per Sample, in the Batch Iterative Processing container.
 - Creates a report for each sample, that contains the results from the *Peptide Mapping* activity nodes, before review.
- 2. Export Summary Report, out of the Batch Iterative Processing container.
 - Creates a report that contains the sequence coverage results for all samples from the *Review Results* activity node and any warnings from the workflow.
- To create individual reports of reviewed results:
 - 1. Use either Save Annotations Snapshot in the Batch Iterative Processing container, or Save Review Snapshot out of the Batch Iterative Processing container to save Peptide Mapping results.
 - 2. Review the sbf files separately in the Pepmap_ReviewSnapshots workflow.
 - 3. Use *Export PDF Report* in the Pepmap_ReviewSnapshots workflow to create one report per sample.

6. Review Snapshots

WORKFLOW SPECIFIC INFORMATION AND GUIDELINES

Review Snapshots Workflow





Pepmap_ReviewSnapshots_Be5.0

- Use this workflow to review saved Snapshot results that have peptide annotations.
 - For example, to review individual results
 Snapshots from the Batch Processing
 workflow and create a report.

Review Saved Results



Load Results	Snapshots - Settings	
General Advan	ced Display	
Name: Format:	Pepmap Review Auto Detect	~
Files/Folders:	20210901_NISTMAb_TimeCourse_Control_4ul_EAD_1.sbf 20210901_NISTMAb_TimeCourse_Control_4ul_EAD_2.sbf 20210901_NISTMAb_TimeCourse_Control_4ul_EAD_3.sbf	

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~×	Range	Peptide	Modifications	Flags				
×	165 HC[278-291]	FNWYVDGVEVHNAK	Phe->Gln	R				
X	176 HC[278-291]	FNWYVDGVEVHNAK	Asn->Glu	R				
~	256 HC[396-412]	TTPPVLDSDGSFFLYSK	Ser->Asn	A				
~	362 LC[169-182]	DSTYSLSSTLTLSK	Ser->Asn	A				
				_				
\checkmark	375 LC[190-206]	VYACEVTHQGLSSPVTK	Carbamidomethyl,	A				
	1 HC[1-5]	QVTLR	Gln->pyro-Glu					
	2 HC[1-18]	OVTI RESCRAI VKPTOTI	Gln->nvro-Glu					

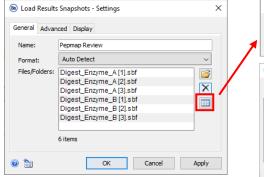
- When multiple samples are analyzed in the other Peptide Mapping workflows, each sample creates its own sbf file.
 - To load saved sbf files into the Pepmap_ReviewSnapshots workflow, select all of the individual sbf files in the parent folder.
 - Data will not load if the parent folder is selected.

- The *Review Results* activity node opens a copy of the previous analysis.
 - Any previously accepted or rejected peptides have the applicable entry in the Flags column.
 - A further review is then possible.
 - The reviewed sbf files and a new report can be saved.

Load Snapshots: Metadata



- Special Use Case: To combine samples with different sample preparation methods that have been processed in different Peptide Mapping workflows.
 - For example: Samples of the same molecule that have been digested with different enzymes, and then analyzed separately using a Peptide Mapping workflow.
 - 1. On the General tab, click the icon to open the Metadata Editor.
 - 2. Add a column called Data Set.
 - Note: Spaces and capitalization are critical. For example, **Data set** will not work.
 - The overall Sequence Coverage takes all of the samples into account.



📵 Metadata Editor				>
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Name Digest_Enzyme_A [1].sbf Digest_Enzyme_A [2].sbf Digest_Enzyme_A [3].sbf Digest_Enzyme_B [1].sbf			Data Set Trypsin Trypsin Trypsin AspN	0
Name Digest_Enzyme_A [1].sbf Digest_Enzyme_A [2].sbf Digest_Enzyme_A [3].sbf Digest_Enzyme_B [1].sbf Digest_Enzyme_B [2].sbf			Data Set Trypsin Trypsin Trypsin AspN AspN	0
Name Digest_Enzyme_A [1].sbf Digest_Enzyme_A [2].sbf Digest_Enzyme_A [3].sbf Digest_Enzyme_B [1].sbf			Data Set Trypsin Trypsin Trypsin AspN	0

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		۲	20210415	Sciex WiffTwo	AspN	1720 :	
,		۸	20210415	Sciex WiffTwo	AspN	1720 :	
		۸	20210415	Sciex WiffTwo	AspN	1720 :	
		۸	20210415	Sciex WiffTwo	Trypsin	1720 :	
		۸	20210415	Sciex WiffTwo	Trypsin	1720 :	
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		۸	20210415	Sciex WiffTwo	Trypsin	1720 :	
		۰	20210415	Sciex WiffTwo	Trypsin	1720 :	

Review Results: Configure Settings





💩 Review Resu	lts - Settings			×
Pepti	de Chromatograms	Report	Display	
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Review Results	- Settings		×
	Chromatograms	Report	Display
Sequence	Modifications	Disulfide	Conjugates
Fixed: Carbamid	omethyl (C)		-
0 🛅	ОК	Cancel	Apply

Sequence tab:

- Sequence(s):
 - If sbf files were saved from data analyzed with Biologics Explorer software 4.0, then select From Data.
 - If sbf files were saved from data analyzed with Biologics Explorer software 3.1 (or earlier versions), then:
 - If all samples have the same sequence, then select **From Text** and type the sequence, or **From Fasta File** and add the applicable file.
 - If different samples require different sequences, then select From Metadata: Fasta File, Sequence IDs (optional).
 - Note: For more information about Batch Processing, refer to the section: B:
 5.Guidelines for Peptide Mapping Batch Processing Workflows.

Modifications tab:

• Select the **Fixed** modifications specified in the previous *Peptide Mapping* activity nodes.



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