Peptide Mapping

Biologics Explorer 2.0 Quick Guide

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Peptide Mapping: Biologics Explorer Quick Guide

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 - Pepmap_Simple
 - Pepmap_Extended
 - Pepmap_Comparative
 - Pepmap_ReviewSnapshots



Part A Software and Workflows

1. OVERVIEW OF APPLICATIONS





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Overview of Applications for Peptide Mapping Workflows

- These workflows are primarily designed for peptide mapping analyses of enzymatically digested biotherapeutic molecules:
 - Sequence coverage and confirmation
 - Glycopeptide analysis
 - Post-translational modification (PTM) analysis
 - Target PTM profiling

- Disulfide-bond (DSB) analysis
- Conjugate analysis
- Sequence variant analysis (SVA)
- Batch analysis of replicate samples of the same molecule is possible:
 - In-depth characterization
 - Comparison of multiple samples: Process development, instrument method development
 - Stress tests
 - Reduced vs. non-reduced sample comparisons



Part A Software and Workflows

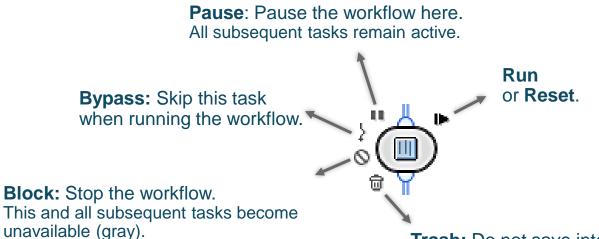
2. USING BIOLOGICS EXPLORER





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ACTIVITY NODE ICONS



Trash: Do not save intermediate data. When this icon is activated, the results for this particular activity node cannot be viewed. Using the Trash icon helps to save memory. Use this feature after workflow settings have been optimized.



Using Biologics Explorer

WORKFLOW ICONS **Workflow Completed** All activity nodes have completed successfully. Workflow Paused Some activity nodes have been completed successfully, but some have not yet started. Active 2. PeptideMapping_Simple **Workflow Ready** 3. PeptideMapping_Extended No activity nodes have been completed. The PeptideMapping_Comparative Analysis workflow is ready to start. 2. PeptideMapping Simple 3. PeptideMapping_Extended Workflow Error Some activity nodes have been completed successfully, but at least one activity node cannot run.

Workflow Warning Some activity nodes are incomplete.



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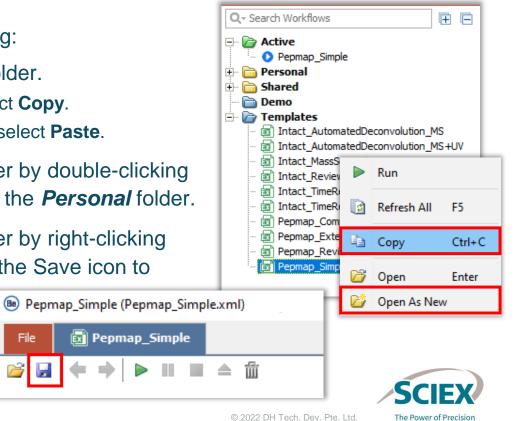
Using Biologics Explorer: General Overview

START AND SAVE WORKFLOWS

To open a workflow, do one of the following:

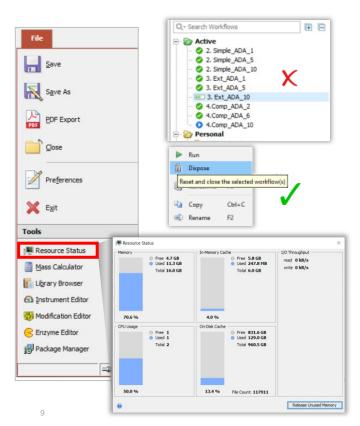
- Copy a workflow from the **Templates** folder.
 - 1. Right-click on the workflow and then select **Copy**.
 - Right-click the **Personal** folder and then select **Paste**. 2.
- Open a workflow in the **Templates** folder by double-clicking and then use the **Save** icon to save it in the **Personal** folder.
- Open a workflow in the *Templates* folder by right-clicking and then selecting **Open As New**. Use the Save icon to save it in the **Personal** folder.

File



Using Biologics Explorer: General Overview

RECOMMENDATIONS FOR CORRECT USE OF THE RESOURCES



- Follow best practices to make sure that Biologics Explorer has sufficient memory and computing power:
 - Only run one workflow at a time: Some activity nodes are very resource intensive. Co-processing might use up all available resources.
 - To conserve memory, activate the Trash icon whenever possible in optimized workflows.
 - After reviewing data and saving results, reset or dispose workflows before starting a new analysis.
 - Use Save Snapshot activity nodes to enable completed results to be saved or reviewed in the Pepmap_ReviewSnapshots workflow.
- The processing computer should have at least 250 GB of free disk space and 6 GB of In-Memory Cache.
 - Files being processed for peptide mapping workflows should not add up to more than 4 GB.



The Power of Precision

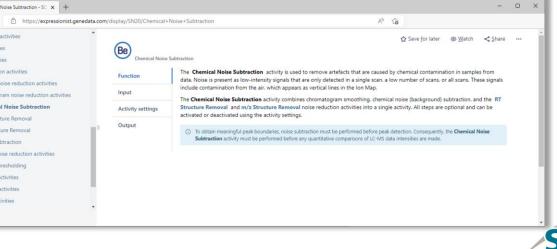
Using Biologics Explorer: General Overview

×

ACCESS THE ONLINE HELP

General Adva		Noise Subtracti	on - Settings
	togram Smoothir	ng	
RT Window: Estimator:	5 Moving Averag	Scans	~
RT Window: Quantile:	al Noise Subtract	ion Scans	Chemical Noise Subtraction - SC C https://exp Identification activities
Method: Threshold:	Clipping (14	Subtraction	 Import activities MS/MS activities Noise reduction activities Spectrum noise reduction activities
0		OK	 Chromatogram noise reduction Chemical Noise Subtraction mz Structure Removal RT Structure Removal Blank Subtraction UV trace noise reduction activit Intensity Thresholding Peak finding activities Quantitation activities Restriction activities

• For information about individual activity nodes and their settings, click the ? icon to view the relevant Help pages.





Part A Software and Workflows

3. GENERAL GUIDELINES FOR PEPTIDE MAPPING WORKFLOWS



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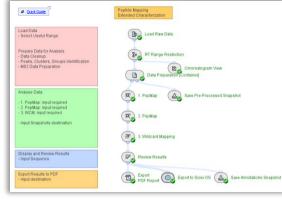
GENERAL GUIDELINES FOR PEPTIDE MAPPING WORKFLOWS

General Peptide Mapping Workflow Guidelines

WORKFLOW TYPES



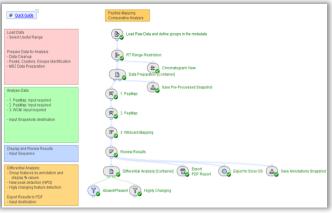
Pepmap_Simple



Pepmap_Extended



Pepmap_ReviewSnapshots



Pepmap_Comparative



General Peptide Mapping Workflow Guidelines

COMMON ACTIVITY NODES IN THE PEPTIDE MAPPING WORKFLOWS

- A. Load Raw Data
- B. RT Range Restriction
- C. Chromatogram View
- D. Data Preparation [Container]
 - i. Chromatogram Chemical Noise Subtraction
 - ii. Chromatogram RT Alignment
 - iii. Chromatogram Peak Detection
 - iv. Chromatogram Isotope Clustering
 - v. Singleton Filter
- E. Peptide Mapping
- F. Review Results
- G. Reporting and Exporting

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



Load Raw Data: Add Analysis Name and Data Files

General tab.

- Type into the Name field to define the analysis.
- Upload raw data files III:
 - Select only wiff or wiff2 container files.
 - When analyzing data from the ZenoTOF 7600 system, use only wiff2 files and not wiff files.
 - Do not select the auxiliary files with the same name.
 - Name
 Display 20210203 Adalimumab tryptic 2ug ECD_1.timeseries.data
 Display 20210203 Adalimumab tryptic 2ug ECD_1.wiff.scan
 Display 20210203 Adalimumab tryptic 2ug ECD_1.wiff2

🐵 Load Raw Da	ata - Settings	×				
General Advan	ced Display					
Name:	Peptide Mapping Extended					
Format:	Sciex WiffTwo (*.wiff2) ~					
	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					

- To view files within a wiff1 or wiff2 container, double-click the wiff or wiff2 container to open it.
 - Choose the files to upload from the list of embedded files.



Load Raw Data: Format

- If individual sample files within a wiff or wiff2 container have the same name, then do not use the **Auto Detect** option.
- To make sure that unique sample names are present in the *Experiment Table*, and that *Review Results* displays the correct quantitative information for each sample:
 - 1. From the Format dropdown list select either Sciex Wiff or Sciex WiffTwo.
 - Only use wiff2 for data acquired using the ZenoTOF 7600 system.
 - 2. Select the Use File Name as Sample Name check box.

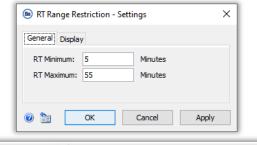


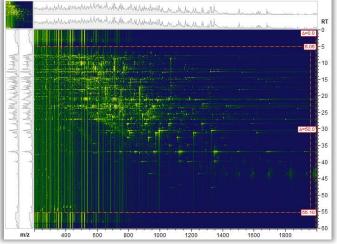
The Power of Precision

Restrict the RT Range

- Run the *Load Raw Data* activity node and then open (double-click) it when data loading is complete.
- Identify the retention time (RT) range where there is meaningful data present.
 - Exclude stray signals caused by valve switching or column wash.
 - Focus on the separation range.

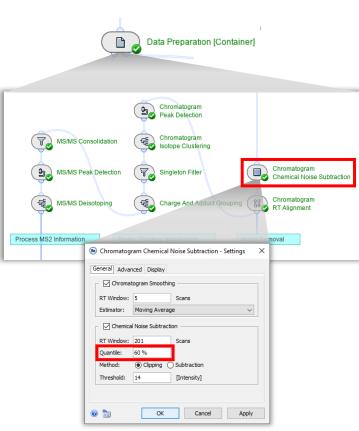
Note: If the fields are blank, then the full RT range is used.



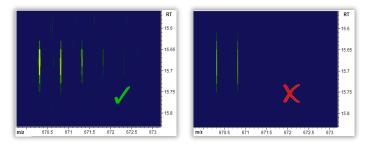




Data Preparation: Chemical Noise Subtraction - Quantile

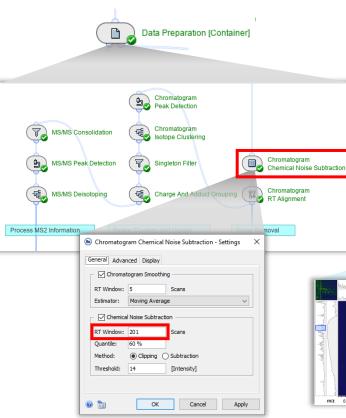


- Change this setting only if the default noise removal is too harsh, which can be identified by:
 - Loss of low intensity isotope peaks from singly (+1) or doubly (+2) charged clusters, or from low intensity clusters of interest.
 - Excessive cutoff of the tails of very wide (extended RT) peaks.

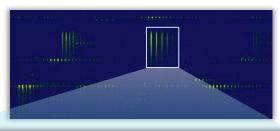


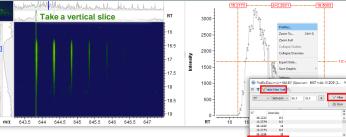
 If clusters of interest are affected, or if the unwanted peak modifications that are described above appear too frequently, then set the Quantile to a lower value, such as 50%.

Data Preparation: Chemical Noise Subtraction - RT Window



- If the largest peak in the dataset contains less than 50 scans, then decrease the **RT Window** (to 101 or 151 scans, for example).
 - As a rule of thumb, the **RT Window** should be at least double the number of scans across the largest peak in the dataset.



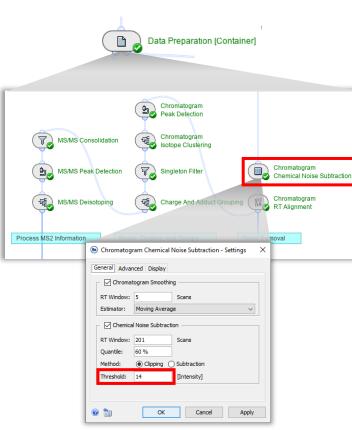


To determine the number of scans:

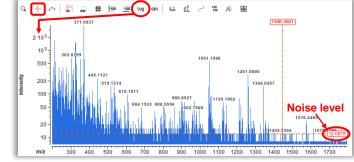
- 1. Locate a feature that extends over a longer RT than other features in the ion map.
- 2. Take a vertical slice to generate an Extracted Ion Chromatogram.
- Right-click in the Extracted Ion Chromatogram window and select Profiles.
- Use the Advanced Filter Tool ▼ to select the RT range for the peak.



Data Preparation: Chemical Noise Subtraction - Threshold

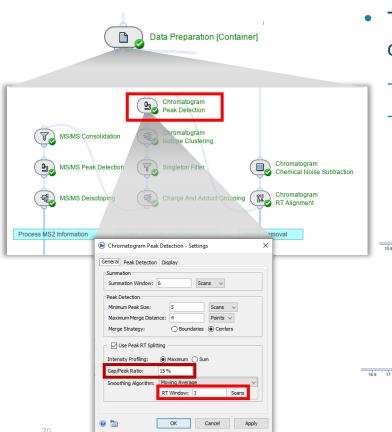


- If the noise level is significantly different from the Threshold value pre-set in the Chromatogram Chemical Noise Subtraction activity, then change this setting.
- To measure the noise level and determine an appropriate **Threshold** intensity value:
 - 1. Expand the mass spectrum intensity axis by dragging it until the noise level is readable, or change the axis from linear to the log scale using the icon in the tool bar.
 - 2. Use the crosshair tool + to measure the intensity of the noise level.





Data Preparation: Chromatogram Peak Detection

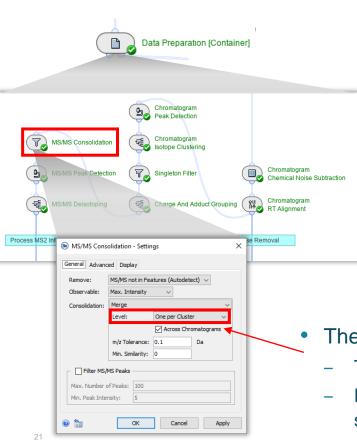


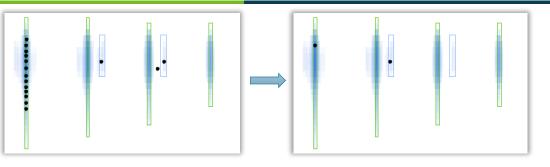
- The peak splitting of closely eluting components in the RT direction can be modified as required.
 - To decrease splitting: Increase the Gap/Peak Ratio.
 - To increase split sensitivity: Decrease or remove **Smoothing**.



GENERAL GUIDELINES FOR PEPTIDE MAPPING WORKFLOWS

Data Preparation: MS/MS Consolidation





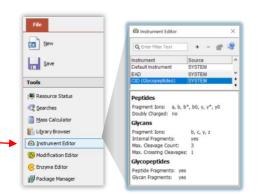
- This activity node merges MS/MS data across equivalent peaks and clusters.
- Consolidation can improve MS/MS spectra, resulting in more identifications.
- Consolidation can reduce false positives if MS/MS spectra are too ambiguous.
- There is an option to merge MS/MS **Across Chromatograms**.
 - This improves confidence in identifications across technical replicates.
 - Do not use this option when assessing individual sample sequence coverage.



Peptide Mapping: Configure Settings (1)

Peptide Mapping	General tab:
Ť	Instrument
1. PepMap - Settings ×	experiment
Peptide Chromatograms Report Display General Sequence Modifications Glycosylation Disulfide Conjugates	 To review
Mass Tolerance: 8 ppm ∨	Instrum
Instrument: EAD v m/z Tolerance: 0.95 Da v	
Min. Score: 80 Keep: Top Ranked ✓ Mass-only Matches: Discard all ✓	• m/z Tolera
Ignore Annotated Features	 The defa
Export Coverage Data (deprecated)	the poss
	MS/MS
	Reduce
OK Cancel Apply	is beir

- t: Select according to specific al set-up.
 - w or modify: Navigate to File > Tools > ent Editor.



nce:

- ault value (0.95 Da) is not a reflection of MS mass accuracy. It increases sible options for identification as a compromise for the potential impact of pre-processing on the m/z.
 - ce the **m/z Tolerance** to 20 ppm when data generated using the ZenoTOF 7600 system ng analysed. The m/z Tolerance can also be reduced to 20 ppm for analysis of data generated using other MS systems.
 - Reducing the m/z Tolerance limits the number of false positives or ambiguous annotations that are generated.
- The default value can be increased if required, for example, if the data has wide error distributions.



Peptide Mapping: Configure Settings (2)

Peptide I	Mapping		Sequ	ence tab:
			• Sec	uence(s):
1. PepMap - Settings Conjugates Pe General Sequence Sequence(s):	>LC DIQMTO >TRY IQVRLG >rLySC MHKRT	YLNACLVLALAAGASQAI	• Enz	Enzyme spe length can b cymes : To v n the Selec
Enzymes:	Trypsin	+		
Max. Missed Cleavages: Min. Peptide Length:	4 5	General Sequence	tide Chromatograms F Modifications Glycosy amidomethyl (C)	Report Display lation Disulfide
0 1	OK	Max Allo Uni	onia-loss (N) dimum: 1 per Sequence wedi: Anywhere modified: is not Required w Glycosylation: true	
		Maa Alic Uni Alic Gin->	nidated (NQ) dimum: 2 per Sequence wwed: Anywhere modified: is not Required w Glycosylation: true pyro-Glu (N-term Q)	Q. Enter Filter Text Deamidated (NQ) Gin->pyro-Glu (N-term Q) Lys-loss (Protein C-term K)
		Sequence Variants: No V Maximum: 3		Codetion (M)
		0	OK Cance	el App

0:

): Paste as text or upload as a FASTA file.

*

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\$7 ☆

Cancel

OK

- pecificity, maximum number of missed cleavages, and minimum peptide n be adjusted as required.
- o view the list of system-configured and user-defined enzymes, ect Entries dialog using the + on the left.

Modifications tab:

- View the list of possible **Fixed** or **Variable** modifications by opening the **Select Entries** dialog using the + on the left.
 - Commonly used modifications can be added as favorites by selecting the star icon.
 - To analyze over- or under-alkylation, set the alkylating reagent to Variable for cysteine and other target amino acids.



Peptide Mapping: Configure Settings (3)

) 1. Pep	Map - Settings		×
Conju Genera		Report Glycosylation	Display Disulfide
Type:	Clycosylated Library: CHO N-Glycans small CHO N-Glycans small CHO N-Glycans small CHO N-Glycans small Second CHO N-Glycans medium User defined Max. Number of Glycans per Peptide: Substituents:	1	~
	Max. Substitutions:	1	

Note: Disabling **Glycan Fragments** for glycopeptides in the **Edit Instrument** settings reduces the time required for complex glycan searches.

It is recommended to have **Glycan Fragments** enabled for data acquired in CID mode.

General Peptides Glycans Glycopeptides
Peptide Fragments: 🔽 Glycan Fragments: 🗌
OK Cancel

Glycosylation tab:

- Library: Select a system-configured or a user-defined library.
 - To review or modify a glycan library: Navigate to File > Tools > Library Browser > Resources.
- Max. Number of Glycans per Peptide: There is a threshold number of Estimated Glycopeptide Candidates allowed for the search to proceed (refer to next page for details).
 - Allowed Sites: Only N-linked: There is typically fewer potential consensus sequences per peptide, so searches for *N*-glycosylation are generally more tolerant of search criteria:
 - Up to 4 *N*-glycans per peptide is the maximum allowed value.
 - The number of missed cleavages and variable modifications impacts the search time.
 - **Allowed Sites: Only O-linked:** Every serine and threonine (S and T) residue is a potential site for *O*-glycosylation.
 - Long peptides containing many potential glycosylation sites dramatically impact the number of Estimated Glycopeptide Candidates, and the subsequent processing time.
 - Using enzymes that result in shorter peptides, for example using Trypsin/P in the settings so that cleavage is <u>not</u> restricted at RP/KP, can help to reduce the search time and limit the total number of candidates.



Peptide Mapping: Configure Settings (4)

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

Enzyme: Trypsin	Glycans/	Size of glycan library				
Missed cleavages: 1	peptide	3	4	5	6	7
U	3	✓	\checkmark	\checkmark	\checkmark	\checkmark
	4	✓	✓	\checkmark	✓	×
	5	✓	✓	×	×	×
	6	×	×	×	×	×
	7	x	x	x	x	x

Enzyme: Trypsin/P Missed cleavages: 0

Size of glycan library					у
Glycans/ peptide	3	4	5	6	7
3	\checkmark	 Image: A start of the start of	\checkmark	 Image: A start of the start of	 Image: A start of the start of
4	✓	\checkmark	\checkmark	\checkmark	✓
5	✓	✓	✓	\checkmark	×
6	✓	 Image: A start of the start of	\checkmark	×	×
7	✓	 Image: A start of the start of	✓	×	×

Glycosylation tab:

- A combination of factors is used to calculate the number of Estimated Glycopeptide Candidates to determine if the search will proceed:
 - The number of glycans in the glycan library (including Substituents).
 - Use the smallest library size that contains the relevant glycans of interest, •
 - Selecting Filter for Core Structures can help to limit the candidates. •
 - The maximum allowed number of glycans per peptide.
 - Do not exceed what is expected for the molecule. Lower values allow larger • libraries to be used, and vice versa.
 - The theoretical sites of glycosylation on a peptide.
 - The number of missed cleavages and the enzyme specificity contribute to this. •
- Other search parameters contribute to the overall search time. ۲
 - To reduce 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. •
 - Reduce the number of glycans per peptide. •
 - Reduce the size of the glycan library. •



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Peptide Mapping: Configure Settings (5)

Peptide Mapping	Peptide Mapping - Settings ×
Υ	Conjugates Peptide Chromatograms Report Disolav General Sequence Modifications Glycosylation Disulfide
	Connectivity: Fixed ✓ Bonds: HC:22+HC:97 ∧ HC:223-LC:213 HC:223-LC:213 HC:223-LC:213 HC:223-LC:223 HC:223-LC:224 ∨ Max. Peptides: 4
Peptide Mapping - Settings General Secuence Modifications Glvcosylation Conjugates Peptide Chromatograms Repo Chromatogram of : All Peaks RT Range: Extended Peak Boundaries	
Intensity Profiling: O Maximum Sum	Peptide Mapping - Settings ×
	General Sequence Modifications Glycosvlation Disulfide
	Conjugates Peptide Chromatograms Report Display
OK Cancel	Show Coverage per Experiment Presence Threshold: 1.0 [% Intensity]
	-Ouantification
	Observable: Volume ~
	Condensing Method: Sum ~
	Chromatogram Thumbnail Size: Medium 🗸
	🛞 🛅 OK Cancel Apply

Disulfide tab:

- For <u>non-reduced</u> samples: Set **Connectivity** to **Fixed**. Specify the expected disulfide bridges using the correct syntax, for example; HC:22-HC:97.
 - The chain names must match those specified in the Sequence tab.
- For <u>reduced</u> samples: Set **Connectivity** to **None**.

Peptide Chromatograms tab:

• These settings control the layout of peptide chromatograms when viewing the results of this activity, and do not need to be changed.

Report tab:

We recommend setting Volume as the Observable for data acquired using a QTOF system.



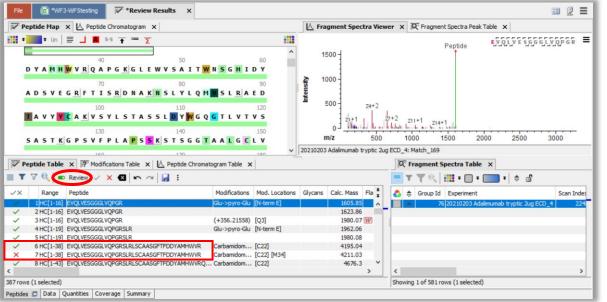
Review Results: Configure Settings

Review Resu) Jifide Conjugates Peptide Chromatograms Report Display	 Sequence tab: Sequence(s): Paste as text or upload as a FASTA file.
Sequence(s):	From Text Sequences:	· · · · · · · · · · · · · · · · · · ·	 Use the same FASTA protein sequences in the Peptide Mapping activity node and the Review Results activity no



Review Results: Review Peptide Mapping Results

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

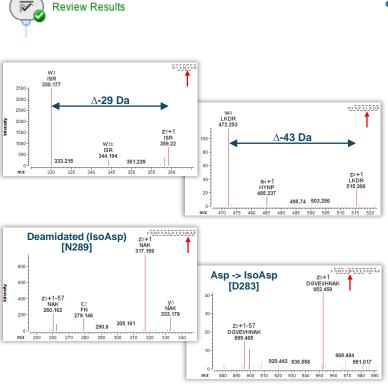


- Activate the **Review** mode and accept one annotation for all relevant peaks.
- 2. Reject all other redundant annotations.
- Click Save to apply the changes. The activity node runs again to automatically recalculate peptide quantities.



GENERAL GUIDELINES FOR PEPTIDE MAPPING WORKFLOWS

Review Results: Isomer Differentiation



Note: Use an **m/z Tolerance** of <20 ppm in the *Peptide Mapping* activity node for optimal results.

- During MS/MS analysis using EAD, diagnostic internal fragment ions are produced that enable differentiation between isomeric amino acid residues.
 - To confirm the presence of leucine (Leu) or isoleucine (IIe):
 - Ions are annotated in the MS/MS spectra as w_n or w_{nb}.
 - Leucine: A \mathbf{w}_{n} ion at a 43 Da mass shift from the corresponding z+1 ion.
 - Isoleucine: A w_n ion at a 29 Da mass shift from the corresponding z+1 ion.
 - To confirm the presence of aspartic acid (Asp) or isoaspartic acid (IsoAsp):
 - Ions are annotated in the MS/MS spectra as c_n+57 or z_m+1-57 .
 - c_n +57 or z_m -+157 ions annotated in the MSMS spectra signify the presence of isoaspartic acid.
 - Aspartic acid does not generate these diagnostic internal fragment ions because there is no methylene group in the peptide backbone.



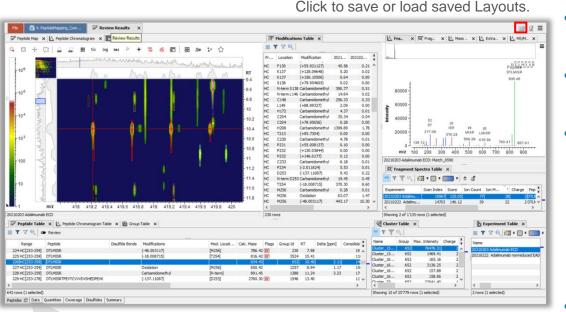
Review Results: Isomer Differentiation (Cyclization and Deamidations)

	Review Results						
📝 Pe	eptide Table 🗙 📝	Modifications Table 🛛 🗙 Peptide Chromatogram Tal	ble x				
	🝸 🔍 💽 Review	$\checkmark \times \boxtimes \bowtie a \blacksquare :$	I				
√X	Range	Peptide	Disulfide Bonds	Modifications	Mod. Locat		
	39 TRY[45-54]	LSSPATLNSR					
	40 TRY[55-62]	VATVSLPR					
		FNWYVDGVEVHNAK		Asp->IsoAsp	[D283]		
		LTVDKSR		Asp->IsoAsp	[D416]		
	32 LC[45-52]	LLIYDTSK		Asp->IsoAsp	[D49]		
	8 HC[259-277	TPEVTCVVVDVSHEDPEVK=VVSVLTVLHQDWLNGKEYKCK	HC:264->HC:324	Deamidated	[N318]		
	12 HC[278-291]	FNWYVDGVEVHNAK		Deamidated	[N289]		
	11 HC[278-291]	FNWYVDGVEVHNAK		Deamidated (IsoAsp)	[N279]		
	13 HC[278-291]	FNWYVDGVEVHNAK		Deamidated (IsoAsp)	[N289]		
	17 HC[305-320]	VVSVLTVLHQDWLNGK		Deamidated (IsoAsp)	[N318]		
	18 HC[305-320]	VVSVLTVLHQDWLNGK		Deamidated (IsoAsp)	[N318]		
	20 HC[305-323]	VVSVLTVLHQDWLNGKEYK		Deamidated (IsoAsp)	[N318]		
	21 HC[305-323]	VVSVLTVLHQDWLNGKEYK		Deamidated (IsoAsp)	[N318]		
	22 HC[305-323]	VVSVLTVLHQDWLNGKEYK		Deamidated (IsoAsp)	[N318]		
	14 HC[292-304]	TKPREEQYNSTYR		G0F	[N300]		
	15 HC[292-304]	TKPREEQYNSTYR		G1F	[N300]		
	7 HC[252-258]	DTLMISR		Oxidation	[M255]		
<							

- To simplify data review, it is possible to filter for relevant modifications. For example:
 - $Asp \rightarrow IsoAsp$
 - Deamidated
 - Deamidated (IsoAsp)
 - The diagnostic internal fragment ions present in the MS/MS spectra can then be used to validate identifications, and results Accepted or Rejected as required.



Review Results: Create Custom Layouts



den Data 📁 Q.

Click to disconnect the Data tab window.

 Each pane can be disconnected and docked at a new location.

- The location where the disconnected pane will be docked is highlighted by a blue box.
- To move the Ion Map into the Peptides tab:
 - 1. Select the **Data** tab and click the icon to disconnect the **Data** tab window.
- 2. The ion map, or any pane from the **Data** tab window, can then be undocked and dragged to a new location on the **Peptides** tab.
- Favorite layouts can be saved and accessed with the Layout icon.



Reporting: Define Destination for Snapshots and Reports

- Select the folders in which results will be stored.
- Two types of results are stored:
 - Snapshots:
 - Snapshots are intermediate results saved permanently as sbf files.
 - Snapshots saved from pre-processed data store the intermediate results generated after Chromatogram Chemical Noise Subtraction and Chromatogram RT Alignment.
 - Snapshots saved after *Review Results* store all of the intermediate information, including annotations of features.
 - PDF Reports, which embed:
 - A PDF document.
 - An Excel file with spectral information from deconvolution.
 - An Executed workflow (xml file), that includes the settings used to generate results.
 - To open the xml file, drag and drop it into the Biologics Explorer workflow home page.

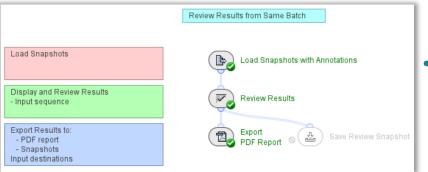
Bave Pre-Process	ed Snapshot - Settings	×
General Display		
Define Destin	ation	
Export Folder:	PepmapSimple Pre-processed	
Export as Fold	er	
0 1	OK Cancel	Apply

Bave Annotations Snapshot - Settings	×
General Display	
Define Destination	
Export Folder: PepmapSimple Annotated	
Export as Folder	
🛞 🛅 OK Cancel Apply	



Reporting: Save Intermediate Results

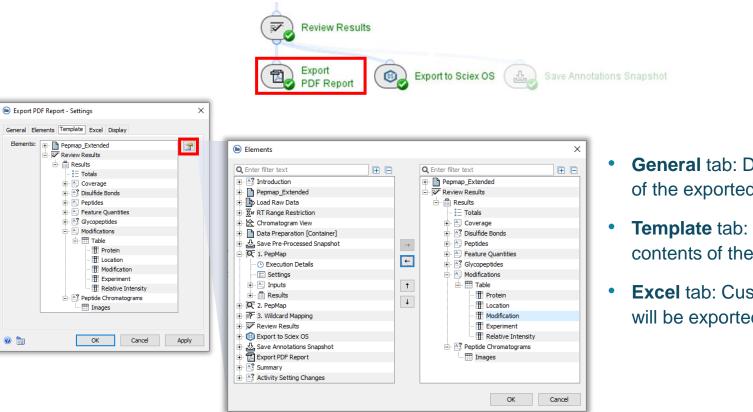




- Store intermediate results after different activity nodes through-out the workflows.
 - Save Snapshot activity nodes create a file (sbf) that contains the required properties of the processed data.
- To review stored data, open sbf files in the *Load Snapshots* activity nodes in the Pepmap_ReviewSnapshots workflows.
 - Load snapshots from Save Annotations Snapshot to review results from the same dataset.
 - Load snapshots from Save Pre-processed Snapshot to analyze results from different batches.
- Saved sbf files can also be loaded into the *Load Raw Data* activity node in any workflow.
 - Load snapshots from Save Annotations Snapshot and bypass all activity nodes except for those required for peptide mapping to add more searches to an analysis.



Reporting: *Export PDF Report*



- **General** tab: Define the destination of the exported report.
- **Template** tab: Customize the contents of the report.
- **Excel** tab: Customize the tables that will be exported with the report.



Reporting: Export to SCIEX OS



(B) Export to SCIEX OS - Setting	x z
General Conjugates Display	
Define Destination	
Export File: Reviewed Re	esults from Extended
Clusters per Group To Export:	3
Mass Mode:	Monoisotopic O Most Abundant Isotope
Consider Adducts:	
Observable:	Volume ~
0 1	OK Cancel Apply

• General tab:

- Define the destination of the reviewed results.
- Define the requirements of the export, for example, the required number of clusters for each group.
- The *Export to SCIEX OS* activity node should <u>not</u> be used in combination with the *Wildcard Mapping* activity node.
 - The *Wildcard Mapping* activity node can be bypassed in relevant workflows.

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.



Part B Workflows and Applications

GUIDELINES FOR SPECIFIC PEPTIDE MAPPING WORKFLOWS



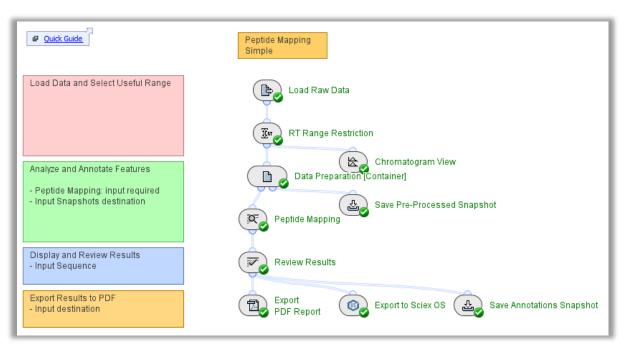
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Simple Peptide Mapping WORKFLOW SPECIFIC GUIDELINES



C IT IS NOT THE

Simple Peptide Mapping Workflow: Design



Pepmap_Simple



Simple Peptide Mapping Workflow: Overview

 \times

fide

Peptide Mapping	- Settings		×			
eptide Mapping	- settings					
Conjugates General Sequence	Peptide Chromatograms Modifications Glycos		Display Disulfide			
Fixed:	Carbamidomethyl (C)		+			
Variable:	Deamidated (NQ) Maximum: 2 per Sequence Allowed: Anywhere Unmodified: is not Required Allow Glycosylation: true Gln->pyro-Glu (N-term Q) Maximum: 1 per Sequence Allowed: Anywhere		+			
	Unmodified: is not Required Allow Glycosylation: true	(B) Peptic	le Mapping - Setti	ngs		
San Jana Varianta	Lys-loss (Protein C-term K)	Conjug General		Chromatogram Iodifications	s Report Glycosylation	Disp Disu
Sequence Variants: Maximum:	4 per Peptide	Type:	Glycosylated			
	, per epuie		Library: CHO N-	Glycans small		~
0 🛅	OK Cance		Allowed Sites:		Only N-Link	ked 🗸
			Use Consensus Se	quences:	\checkmark	
			Filter for Core Stru	ictures:	\checkmark	
			Max. Number of G	lycans per Pepti	de: 1	
			Substituents:			+
			Max. Substitutions		1	
			_			
		0 🛅		ОК	Cancel	App

- Use this workflow when completing routine analyses with non-complex biotherapeutic molecules.
- The combination of search parameters in the *Peptide Mapping* activity node identifies commonly expected peptides and modifications, including glycosylation.



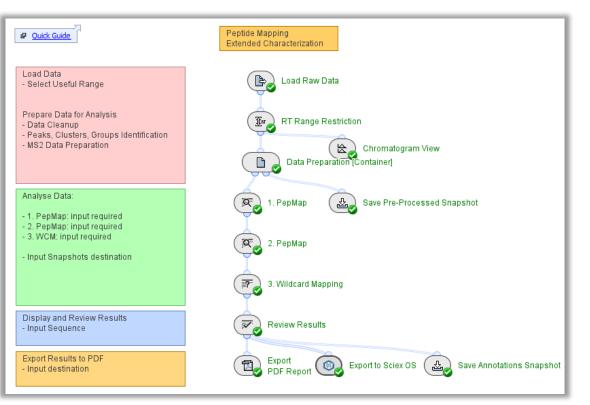
Extended Peptide Mapping

WORKFLOW SPECIFIC GUIDELINES



C. Transaction

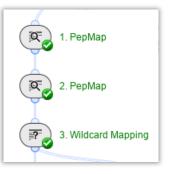
Extended Peptide Mapping Workflow: Design



Pepmap_Extended



Extended Peptide Mapping



🐵 2. PepMap - Setting	gs ×				
	de Chromatograms Report Display Modifications Glycosylation Disulfide				
Mass Tolerance: 8	ppm 🗸				
MS/MS Identifica	ation				
Instrument:	EAD \checkmark				
m/z Tolerance:	0.95 Da 🗸				
Min. Score:	80				
Keep:	Top Ranked 🗸				
Mass-only Matches:	Discard all \sim				
☐ Ignore Annotated Features ☐ Export Coverage Data (deprecated)					
0 🛍	OK Cancel Apply				

- For a more comprehensive peptide mapping analysis, results from up to three consecutive activity nodes can be combined to extend the search space while minimizing false positives:
 - 1. РерМар
 - Identifies the most expected peptides and modifications.
 - 2. РерМар
 - Digs deeper into the sample.
 - Ignore Annotated Features: Makes sure that only unannotated features from the previous search are considered.

3. Wildcard Mapping

 Searches for unexpected modifications, which can be subsequently added to 1.PepMap or 2.PepMap activity nodes.

Note: For biotherapeutics with expected *N*- and *O*-glycosylation, false positives are reduced when *1.PepMap* is used to identify expected *N*-glycans, and *2.PepMap* focuses on the typically less well characterised *O*-glycans.



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Step-Wise Peptide Mapping: Application Examples

R	1. РерМар
R	2. РерМар
7	3. Wildcard Mapping

Three consecutive peptide mapping steps can be combined, depending on the type of analysis required.

Example 1: Disulfide bond (DSB) analysis for non-reduced samples.

Key settings specific to this type of analysis:

 Be 1. PepMap - Conjugates General Set 	Peptide Chr	romatograms Rep fications Glycosylatio	port Display ion Disulfde	
Connectivity:	Fixed		🐵 2. PepMap - Settings	×
0	Bonds: Max. Peptides:	HC:22-HC:96 HC:148-HC:204 HC:224-LC:214 HC:230-HC:230 HC:233-HC:233 HC:265-HC:325 4 K Cancel	Conjugates Peptide Chromatograms Report Disola General Sequence Modifications Glycosylation Disolfid Connectivity: Scrambled Bonds:	
			@	

1. **РерМар**

- Sequence tab: Enzyme Fully specific.
- Disulfide tab: Fixed Connectivity.
 - Define the expected disulfide bridges using the correct syntax (HC:22-HC:96).

2. РерМар

- Sequence tab: Enzyme Fully specific.
- Disulfide tab: Scrambled Connectivity.

3. Wildcard Mapping

On All Peptide Candidates for more annotations relating to unknown modifications.



	1. PepMap 2. PepMap 3. Wildcard Mappir	• T th Ex
💩 2. PepMap - Sett	ings	×
Conjugates General Sequence	Peptide Chromatograms e Modifications Glyc	Report Display cosylation Disulfide
Fixed:		+ ^
Variable:	Ovidation (M)	<u>^</u> +

hree consecutive peptide mapping steps can be combined, depending on ne type of analysis required:

ample 2: Post translational modification (PTM) analysis.

Key settings specific to this type of analysis:

difications Glycosylation Disulfide			2. PepMap - Settings		×
Choonatorares Report Display diffications Glycosylation Disulfide Image: Anywhere additions is not Required (Gycosylation) true (Synumenin (W)) Image: Anywhere additions is not Required (Gycosylation) true (Gycosylation) true (Gycosylation) true (Gycosylation) true (Gycosylation) true (Gycosylation) true (Gycosylation) true (Gycosylation) Image: Gycosylation (Gycosylation) true (Gycosylation) t	gs	×	General Sequence	Modifications Glycos	
ion (M) num: 2 per Sequence ned: Anywhere doffed: s not Required (Disulfide		Sequences: >HC EVQLVES	
ion (M) mum: 2 per Sequence edi Anyuhere odified: is not Required if Oycosylation: true (Vgruenin (W) mum: 1 per Sequence edi Anyuhere odified: is not Required if Oycosylation: true (OK Cancel Apply inants Glu->pyro-Glu Oxidation Trp->Kynurenin		+	-	×	
Caycosystem true Cayco	Maximum: 2 per Sequence Allowed: Anywhere Unmodified: is not Required			^	Ň
Apply OK Cancel Apply	Trp->Kynurenin (W) Maximum: 1 per Sequence Allowed: Anywhere Unmodified: is not Regured		SemiArg-C	Cancel	
Glu->pyro-Glu Oxidation Trp->Kynurenin			0	OK Cancel	Apply
	nor Dootido	~	×		

РерМар

- Sequence tab: Enzyme Fully specific.
- **Modifications** tab: Abundant and expected modifications.

РерМар

- Sequence tab: Enzyme Semi-Specific.
- **Modifications** tab: Shorter list of expected modifications.
- Dr:
- Sequence tab: Enzyme Fully specific.
- Modifications tab: Alternative set of less common modifications that might be expected at low abundance.

Wildcard Mapping

On All Peptide Candidates for annotations relating to unknown modifications.



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Sequence Varian

Properties

GIn->D

Ammon

2. PepMap	E	xample 3:	Seq
3. Wildcard Mappi	• ng	Key settir	ngs
[🐵 1. PepMap - Setti	ngs	×
2. PepMap - Settings Consumates Peptide Chromatograms Re General Sequence Modifications Glycosyla Mass Tolerance: 5 ppm v MS/MS Identification Instrument: EAD m/z Tolerance: 20 ppm v	Conjugates P General Sequence Fixed: Variable:	Carbamidonethyl (C) Gin->pyro-Glu (N-term Q) Maximum: 1 per Sequence Allowed: Anywhere Unmodified: is not Required Allow Givcsylation: true or	Display Disulfide
Min. Score: 80 Keep: Top Ranked Mass-only Matches: Discard all Ignore Annotated Features Export Coverage Data (deprecated)	Sequence Variants: Maximum:	Maximum: I per Sequence Allowed: Anywhere Unmodified: is not Required Allow Glycosylation: true All Substitutions 3 per Peptide Advanced Properties	×
CK Cancel	Apply		incel

1. PepMap

Three consecutive peptide mapping steps can be combined, depending on the type of analysis required:

Example 3: Sequence Variant Analysis (SVA).

Key settings specific to this type of analysis:

1. **РерМар**

- Sequence tab: Enzyme Fully specific. No missed cleavages.
- Modifications tab: Fixed alkylation (cys) with variable modifications limited to the predominant form (for example, pyro-glutamation). Sequence Variants - All Substituents (Restrict Matches cleared).

2. РерМар

- General tab: Low Mass Tolerance. Clear Ignore Annotated Features.
- Sequence tab: Enzyme Semi-Specific. 1-2 missed cleavages.
- **Modifications** tab: Variable alkylation on commonly modified amino acids to detect overalkylation, with all other expected variable modifications.

3. Wildcard Mapping

- On Only Annotated Peptides.
 - Use Review Results to compare additional annotations on the same features to rule out false positives.
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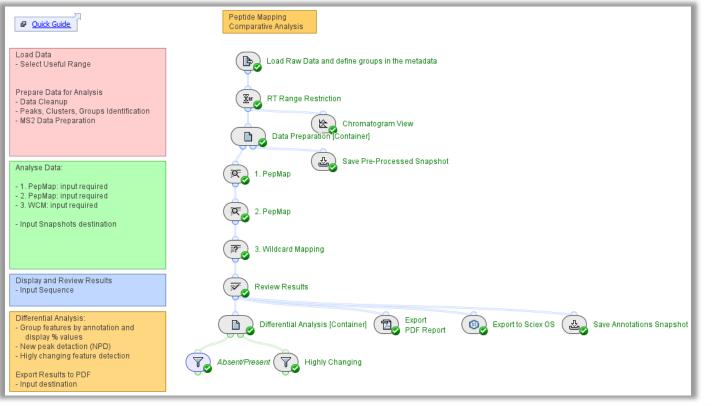
Comparative Peptide Mapping

WORKFLOW SPECIFIC GUIDELINES



The second second

Comparative Peptide Mapping Workflow: Design







Comparative Peptide Mapping



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

- The activity nodes connected with green lines contain statistical tools.
- These statistics activity nodes can be used to compare two datasets, and report peptides that are either:
 - Absent in one sample set but present in the other.
 - Have a specified fold-change difference between sample sets.
 - Example use cases:
 - To compare reduced and non-reduced samples.
 - To compare stressed and unstressed samples.
 - To compare a reference sample with samples from a new batch.



GENERAL GUIDELINES FOR COMPARATIVE PEPTIDE MAPPING WORKFLOWS

Load Raw Data and Define Groups

💩 Load Raw Da	ata and define groups in the metadata - Settings		×	-					
General Advan	nced Display								
Name:	PepMap Comparative Analysis								
Format:	Auto Detect		\sim						
Files/Folders:	INallie	Group Color	^ 📔						_
	20210223 Adalimumab nonreduced 6ug EAD_2.wifl 20210203 Adalimumab tryptic 2ug ECD_2.wiff2	2 NonRed #de6d50 Red #286dde	X		Metadata Editor				
			÷		💕 🖉 🔊 🤊 🖌 👗 🛍 🖉 🐳 🛱	Fi F?	T ×		(
	<	>			Name	Group	Description	Color	Т
	2 items				20210223 Adalimumab nonreduced 6ug EAD_2.wiff2	NonRed			- ·
					20210203 Adalimumab tryptic 2ug ECD_2.wiff2	Red			
0 🛅	0	Cancel	Apply				-		
							OK	Cance	el

- On the General tab, click the table icon to open the Metadata Editor.
 - Specify the **Group** names for the files to be compared.
 - Optionally, add a **Color** column and define colors for each group.



R	1. РерМар
R	2. РерМар
7	3. Wildcard Mapping

 Three consecutive peptide mapping steps can be combined, depending on the type of analysis required:

Example 1: DSB analysis for comparing reduced and non-reduced samples.

Key settings specific to this type of analysis:

 B 1. PepMap - Conjugates General Set 	Peptide Chr	romatograms Rep fications Glycosylatio		
Connectivity:	Fixed Bonds: Max. Peptides:	HC:22-HC:96 HC:148-HC:204 HC:224-LC:214 HC:230-HC:230 HC:233-HC:233 HC:265-HC:325 4	2. PepMap - Settings Conjugates Peptide Chromatograms Report General Sequence Modifications Glycosylation Connectivity: Scrambled Bonds:	X Disolav Disulfide
•		K Cancel	© 😭 OK Cancel	Apply

1. РерМар

- Sequence tab: Fully specific.
- Disulfide tab: Fixed Connectivity.
 - Define the expected disulfide bridges using the correct syntax (HC:22-HC:96).
- **Modifications** tab: Variable alkylation (cys).

2. РерМар

- Sequence tab: Fully specific.
- Disulfide tab: Scrambled Connectivity.

3. Wildcard Mapping

 On All Peptide Candidates for more annotations relating to unknown modifications.
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•

R	1. РерМар
R	2. РерМар
7	3. Wildcard Mapping

	B 2. PepMap - Settings	×
(B) 2. PepMap - Settings X	Conjugates Pentide Chromatograms Report General Sequence Modifications Glycosylation	Display Disulfide
Conjugates Peptide Chromatograms Report Display General Sequence Modifications Glycosylation Disulfide	Sequence(s): From Text Sequences: >HC EVQLVESGGGLVQF	^ ^
Fixed:	Select Entries X SLSAS G Enter Filter Text	
Variable: Oxidation (M) Maximum: 2 per Sequence Allowed: Anywhere Unmodified: is not Required Allow Glycosylation: true Trp->Kynurenin (W) Maximum: 1 per Sequence Allowed: Anywhere Unmodified: is not Persured Allow Glycosylation: true	□ v802	+ - ~
Sequer Se	OK Cancel	Apply
Imperiors Imperiors Gln->pyro-Glu Oxidation Ammonia-loss Carbamidomethyl Deamidated	Trp->Kynurenin	

Three consecutive peptide mapping steps can be combined, depending on the type of analysis required:

Example 2: Comparative analysis for stress testing and lot-to-lot variability.
Key settings specific to this to this type of analysis:

1. РерМар

- Sequence tab: Fully specific.
- Modifications tab: Abundant and expected modifications.

2. РерМар

- Sequence tab: Unspecific enzyme.
- **Modifications** tab: Shorter list of expected modifications. Or:
- Sequence tab: Fully specific.
- **Modifications** tab: Alternative set of less common modifications that might be expected at low abundance.

3. Wildcard Mapping

 On All Peptide Candidates for more annotations.
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~	E.	xample 3:	34/
3. Wildcard Mappi	ng	Key settir	ngs
	🐵 1. PepMap - Settir	ngs	×
2. PepMap - Settings	Conjugates Pe General Sequence	Modifications Glycosylation	Display Disulfide
Conjugates Peptide Chromatograms Re General Sequence Modifications Glycosyla	Fixed:	Carbamidomethyl (C)	+
Mass Tolerance: 5 ppm v MS/MS Identification Instrument: EAD	Variable:	Gln->pyro-Glu (N-term Q) Maximum: 1 per Sequence Allowed: Anywhere Unmodified: is not Required Allow Glycosylation: true	+
m/z Tolerance: 20 ppm v Min. Score: 80 Keep: Top Ranked		Glu->pyro-Glu (N-term E) Maximum: 1 per Sequence Allowed: Anywhere Unmodified: is not Required Allow Glycosylation: true	
Mass-only Matches: Discard all	Sequence Variants: Maximum:	All Substitutions	- F
	0 1	Advanced Properties Restrict Matches	×
🛞 🛅 OK Cancel	Apply	Allow Partial Substitutions	incel

1. PepMap

Three consecutive peptide mapping steps can be combined, depending on the type of analysis required:

Example 3: SVA for comparing wild type (*WT*) and mutant samples.

Key settings specific to this type of analysis:

1. РерМар

- Sequence tab: Enzyme Fully specific. No missed cleavages.
- Modifications tab: Fixed alkylation (cys) with variable modifications limited to the predominant form (for example, pyro-glutamation). Sequence Variants - All Substituents (Restrict Matches cleared).

2. РерМар

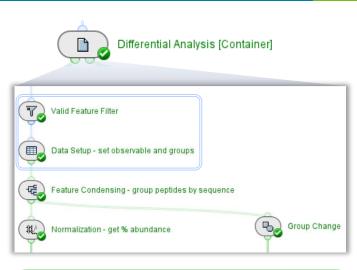
- General tab: Low Mass Tolerance. Clear Ignore Annotated Features.
- Sequence tab: Enzyme Semi-Specific. 1-2 missed cleavages.
- **Modifications** tab: Variable alkylation on commonly modified amino acids to detect overalkylation, with all other expected variable modifications.

3. Wildcard Mapping

- On Only Annotated Peptides.
 - Use Review Results to compare additional annotations on the same feature to rule out false positives. © 2022 DH Tech. Dev. Pte. Ltd.



Differential Analysis Activity Node Settings



- Group Change: Calculates relative and fold-change differences between experiment groups.
- If multiple experiment groups are present, then the reported change is the maximum difference between any two groups.

- *Valid Feature Filter*: Removes any features below a set threshold, and those present in less than a set % or number of experiments.
- This filtering removes insignificant differences or any signal due to noise or artifacts. If expected peptides are not present, optimize this setting.
- Data Setup: Prepares data in a matrix form that can be used as input for filtering and statistics tasks.
 - If Groups were not set in *Load Raw Data*, they can be defined here.
 - Feature Condensing: Groups features based on their annotations.
- Computes a single intensity value for each of the created groups.
- *Normalization*: Provides a comparable basis for further statistical analysis.
- Percent Abundance: Values are summed across all members of each group for each experiment. Each value is divided by the sum from the corresponding group and multiplied by 100.

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Differential Analysis: Data Preparation

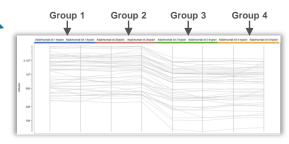
🐵 Data Setup - se	et observable and groups - Settings	×
General Display		
Export:	Auto Detect Observable: Volume	~
Group:	Use Existing	~
	Keep Duplicated Feature Annotations	
Transformation:	None (Keep zero values)	\sim
0 🛅	OK Cancel	Apply

Beature Condensin	g - group peptides by sequence - Settings	×
General Display		
Group by:	Protein Peptide Modifications	
Condensing Method:	Sum V Keep Ungrouped Keep Duplicated Feature Annotations	
0	OK Cancel Apply	

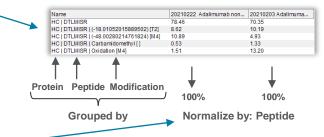
		_
Normalization	n - get % abundance - Settings	×
General Display		
Normalization:	Percent Abundance Group by: Peptide	_
0	OK Cancel Appl	y

- Define Groups in the *Load Raw Data* activity node by editing the Metadata table (preferred option).
- If required, define Groups in the *Data Setup* activity node, using **Group: Manually** and assigning each sample to a group.
- Feature Condensing: Combines features based on existing annotations and calculates a single intensity value (sum, average, median, or max) for each group. For example, the same peptide, from the same protein, with the same modification will be summed using these settings.
 - Run the Data Setup activity node <u>before</u> editing the Feature Condensing settings.
- Normalization: This will report Percent Abundance of condensed features relative to the selected type of annotation. For example, if Group by is set to Peptide, then the % abundance is calculated per peptide. If Group by is left blank, then the % abundance is calculated across the whole sample.

Lot-to-lot comparability



DSB analysis





New and Highly Changing Feature Detection

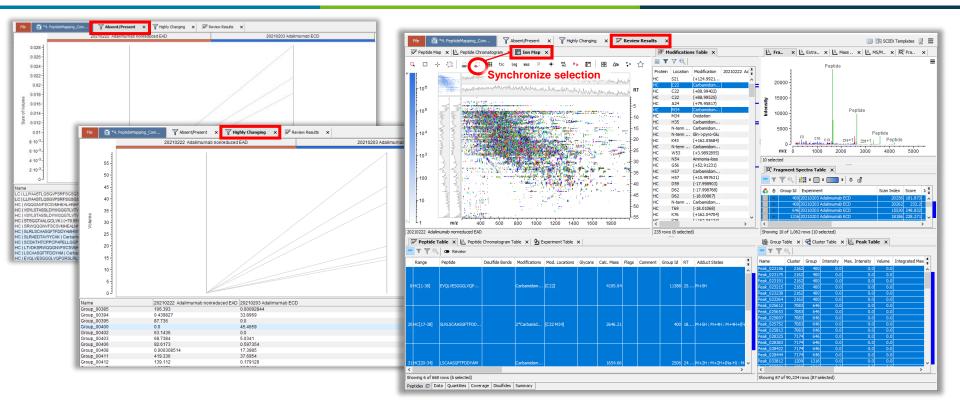
	Co. church com	parative: Absent/Present			×
		20210222 Adalimumab nonreduced EAD	20210203	Adalimumab ECD	
	0.01 8.10 ⁻³ 6.10 ⁻³ In Market M				NonRed
	2.10.3	٢		, ,	Absent Irrelevant Present Absent v in at least 100 % of the experiments
	HC NQVSLTCLV	FDDYAMHWVR=NSLYLQMNSLRAEDTAV (K=SRWQQGNVFSCSVMHEALHNHYTQK (0.0558429 0.0212445 0.0108947	20210203 Adali ^ 0.0 0.0 0.0 0.0	Show relative v counts
Pepmap_Co	mparative (Pepmap_Cor	nparative.xml) [Run]			- 🗆 ×
a + ₽	20210222 Adalims	B es Haja = Hontage View X (È Peside Chronic) umab nonreduced EAD 20210203 Adail		K Frag. × L Extra 1500 1000	L × L MARS. × L MARS. × R MAS. × = NOUSELS STATE
			-17.3 -17.4 至 -17.5 -17.6	20210222 Adamunda Spectra Ta	ble X
781.5	782 782.5 m/z	783 781.5 782 782.5 7 m/z		Group Id Experim	ent Scan Index Score Ion Cr
				<	,
wing 2 Ion Map	6			Shawing 2 of 1,062 rows (1 sel	ected)

- The *Absent/Present* activity node can facilitate New Peak Detection (NPD).
 - The sliders are applied in the input window that opens when the activity node is run.
 - Moving the sliders filters the results. For example, for DSB analysis, the desired features are expected to be **Absent** in the reduced sample and **Present** in the non-reduced sample.
- For *Highly Changing Features*, the desired minimum fold change must be set in the activity node settings.

aunio 20000 -	J222 Adalimumab nonre	Y fold change >10 x) ■ III Q Ξ Noce EAD 20210203 Adalimumab ECD	Name 20210222 Adal 20210203 Adal	mumab nonreduc		NO X III III 2 III 2 III 12 III 12 III 12 III 12 III 12 IIII 12 IIIII 12 IIIII 12 IIIIIIII	Highly Changing - Settings General Display Max. Rank:
t.	¢	,	Color by: -None se	lected- v IIII			Max. P-Value:
Name	20210222 Adalimumal	nonreduced EAD 20210203 Adalimumab	Name	Disulfide Bonds	C Group Char	ge Group Change (Absolute)	Min. Change: 10
Group 02955	109.43	0.00	Group_02955	LC:88->LC:23	0.00		Plint change. 10
Group 01847	25.79	0.00	Group_01847	LC:23->LC:88	0.00		
Group_02240	7.18	0.00	Group_02240	LC:23->LC:88	0.00	69	
Group 00020	1246.17	0.22	Group_00020	LC:23->LC:88	0.00	5633.46	
Group_00292	157.25	0.03	Group_00292	LC:23->LC:88	0.00	5141.97	
Group_00198	2871.66	1.82	Group_00198	LC:23->LC:88	0.00	1578.17	🕜 🐂 OK Cancel Apply
Group_00006	1616.91	1.43	Group_00006	LC:23->LC:88	0.00	1131.01	
Group_00120	397.96	0.43	Group_00120	LC:23->LC:88	0.00	925.83	
Group_00049	1267.23	2.88	Group_00049	LC:23->LC:88	0.00	439.58	
Group_00045	13010.14	31.26	Group_00045	LC:23->LC:88	0.00	416.17	
Group_00022	337.01	0.85	Group_00022	LC:23->LC:88	0.00	397.29	
Group_02945	14.23	0.05	Group_02945	LC:23->LC:88	0.00	289.84	
Group_01912	24.60	0.16	Group_01912	LC:23->LC:88	0.01	158.67	
Group_00804	172.31	1.40	Group_00804	LC:23->LC:88	0.01	122.77	
Group_00046	1089.63	9.21	Group_00046	LC:23→LC:88	0.01	118.33	
Group_00116	69.05	1.52	Group_00116	LC:23->LC:88	0.02	45.28	
Group_00150	30.89	0.87	Group_00160	LC:23->LC:88	0.03	35.37	
Group_01803	91.48	0.00	Group_01803	LC:134->LC:194	0.00	60	
Group_09742	3.70	0.00	Group_09742	LC:134->LC:194	0.00	00	
Group_00035	59491.73	13.28	Group_00035	LC:134->LC:194	0.00	4479.89	SCIEX
Group 00225	171 69	0.30	Group 00225	LC 134->LC 194	0.00	581.40	

The Power of Precision

Synchronized Selections for Simplified Data Review



Open all three windows and activate Synchronize selection in Review Results for dynamic linking.



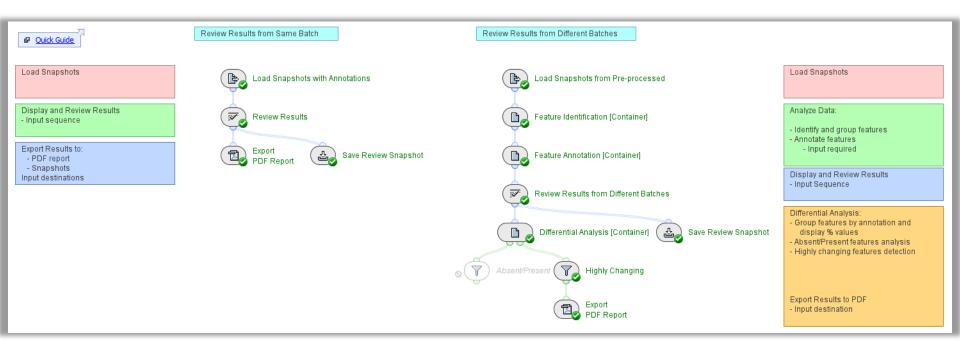
Review Stored Results

WORKFLOW SPECIFIC GUIDELINES



C. Danmar 199

Review Peptide Mapping Snapshots Workflow: Design



Pepmap_ReviewSnapshots



GENERAL GUIDELINES FOR REVIEW SNAPSHOTS WORKFLOWS

Review Results from Same Batch

💩 Load Snapsh	ots from Pre-processed - Settings				×
General Advan	ced Display				
Name:	Review Pre-processed Snapshots				^
Format:	Auto Detect			~	
Files/Folders:	Name	Group	Color	^ 📔	
	20210203 Adalimumab tryptic 2ug ECD_4.sbf	lot1	#286dde	×	
	20210203 Adalimumab tryptic 2ug ECD_5.sbf	lot2	#de6d50	\sim	
	20210203 Adalimumab tryptic 2ug ECD_6.sbf	lot3		¥	
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File System				×
🔇 🌑 🗟 🏂 🏂 Upload Files 🎥 Downl	oad Files) Search		
🛅 Home (Personal)/Pepmap_Extended/snapshots				~
Name	Size	Last Modified	Туре	
20210203 Adalimumab tryptic 2ug ECD_4.sbf	1.4GB	06/22/2022 14:24	SBF	
20210203 Adalimumab tryptic 2ug ECD_5.sbf	1.4GB	06/22/2022 14:24	SBF	
20210203 Adalimumab tryptic 2ug ECD_6.sbf	1.4GB	06/22/2022 14:24	SBF	
File/Folder Name	1.400	Open	Close	y

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

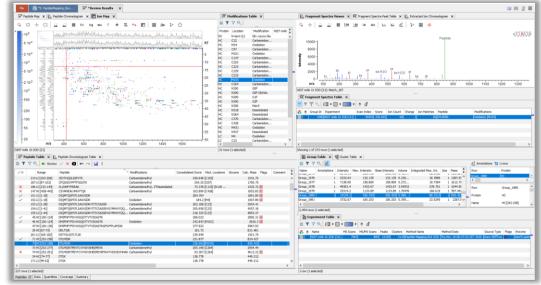


Review Results from Same Batch



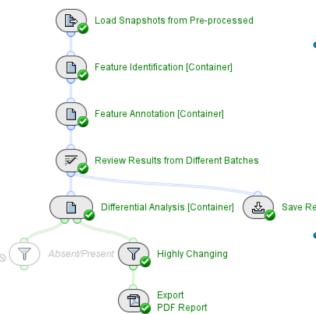
- The Review Results activity node opens a copy of the previous analysis, including any previously accepted and rejected peptides that have the relevant entry in the Flags column.
- A further review is then possible, and the reviewed snapshots can be saved, if required.

 Use this workflow to review previous results from samples that have been analyzed together previously and have peptide annotations.





Review Results from Different Batches



- Data from different analyses can be pre-processed independently and then the Snapshots combined.
- Data requires feature detection including *RT Alignment* and common *Peak Detection*, followed by the *Peptide Mapping* activity nodes for feature annotation.
 - If *Pre-processed Snapshots* are used to process data from the same batch, then bypass the *RT Alignment* activity node.

🐊 Save Review Snapshot

- Comparisons between different groups of samples are also possible.
 - Groups defined in the original workflow are maintained.
 - If not previously defined, groups can be defined within the Load
 Snapshots or the Data Setup activity nodes.





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